Supporting Information

Improvement of DNA Vector Delivery of DOTAP Lipoplexes by Short Chain Aminolipids

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Supporting Results

1. Synthesis of Amino Lipids

1.1 Synthesis of *N*-decyl-*N*-(3-methyoxypropyl)decan-1-amine (AL-A10)

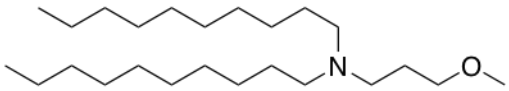

Figure S1: Chemical structure of *N*-decyl-*N*-(3-methyoxypropyl)decan-1-amine (**AL-A10**).

3-Methoxypropylamine (1.00 eq., 5.00 mmol, 0.51 mL), and decanal (2.20 eq., 11.00 mmol, 2.07 mL) were dissolved in DCM (25 mL) and stirred for 3 h. Sodium triacetoxyborohydride (2.20 eq., 11.00 mmol, 2.33 g) was added and the mixture was stirred for 2 days. The product was purified by column chromatography (Silica; EtOAc/MeOH (98:2 V/V) → EtOAc/MeOH (90:10 V/V)) to yield a yellow liquid (702 mg, 1.90 mmol, 38.0 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 3.47 (t, *J* = 5.5 Hz, 2H), 3.33 (s, 3H), 3.14-3.08 (m, 2H), 3.00-2.95 (m, 4H), 2.16-2.09 (m, 2H), 1.82-1.78 (m, 4H,), 1.35-1.27 (m, 28H), 0.89 (t, *J* =7.0 Hz, 6H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ = 69.3, 58.8, 52.4, 50.5, 31.8, 29.2, 29.0, 26.8, 24.1, 23.0, 22.6, 14.1 ppm.

IR $\tilde{\nu}$ = 2924, 2853, 1718, 1579, 1466, 1379, 1256, 1119 cm^{-1}.

HRMS (ESI$^+$): m/z [M+H]$^+$: calculated for $[C_{24}H_{51}NO+H]^+$: 370.4048; found: 370.4037.

R_f (EtOAc / MeOH (9:1 V/V)) = 0.34.

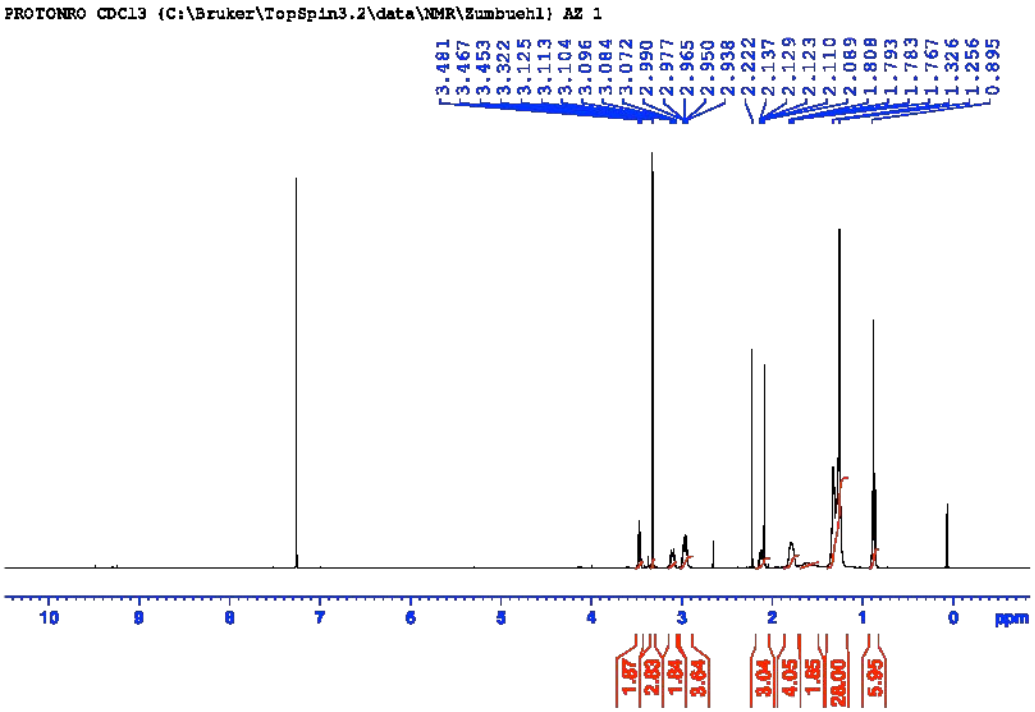

Figure S2: ^{1}H-NMR of *N*-decyl-*N*-(3-methyoxypropyl)decan-1-amine (**AL-A10**).

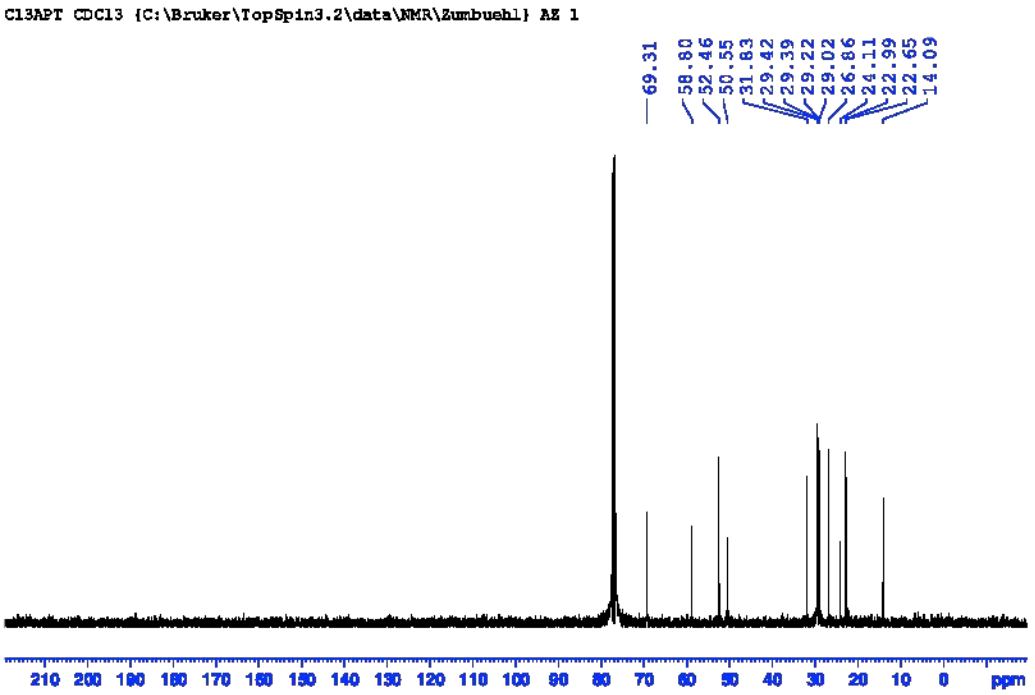

Figure S3: ^{13}C-NMR of *N*-decyl-*N*-(3-methyoxypropyl)decan-1-amine (**AL-A10**).

1.2 Synthesis of *N*-dodecyl-*N*-(3-methoxypropyl)dodecan-1-amine (AL-A12)

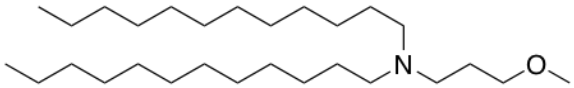

Figure S4: Chemical structure of *N*-dodecyl-*N*-(3-methoxypropyl)dodecan-1-amine (**AL-A12**).

3-Methoxypropylamine (1.00 eq., 5.00 mmol, 0.51 mL), and lauraldehyde (2.20 eq., 11.00 mmol, 2.44 mL) were dissolved in DCM (25 mL) and stirred for 3 h. Sodium triacetoxyborohydride (2.20 eq., 11.00 mmol, 2.33 g) was added and the mixture was stirred for 5 days. The product was purified by column chromatography (silica; DCM/MeOH (98:2 V/V) → DCM/MeOH 90:10 V/V)) to yield a yellow, viscous liquid (588 mg, 1.38 mmol, 28 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 3.49-3.39 (m, 2H), 3.31 (s, 3H), 3.10-3.06 (m, 2H), 2.97-2.93 (m, 3H), 2.05-1.98 (m, 4H), 1.74-1.62 (m, 3H), 1.31-1.25 (m, 36H), 0.87 (t, *J* = 6.9 Hz, 6H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ = 69.5, 58.7, 52.0, 50.1, 31.9, 29.6, 29.6, 29.5, 29.3, 29.1, 26.8, 24.1, 23.0, 22.6, 14.1 ppm.

IR $\tilde{\nu}$ = 2923, 2823, 1717, 1567, 1466, 1379, 1251, 1118 cm^{-1}.

HRMS (ESI$^+$) : m/z [M+H]$^+$: calculated for $[C_{28}H_{59}NO+H]^+$: 426.4674; found: 426.4661.

R_f (DCM / MeOH (9:1 V/V)) = 0.61.

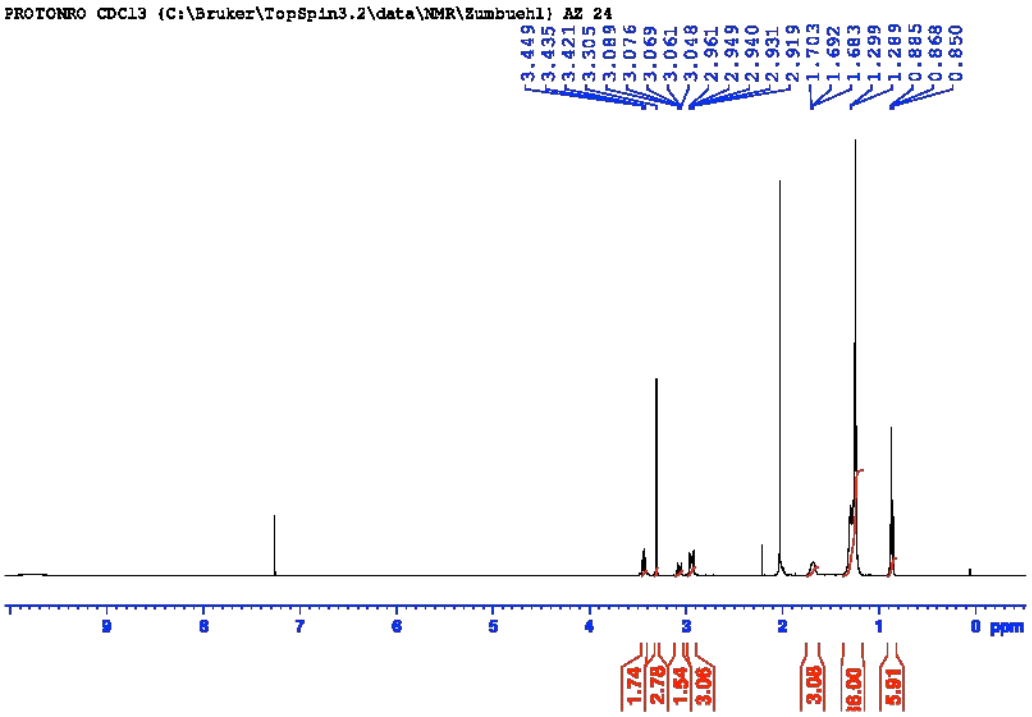

Figure S5: ^{1}H-NMR of *N*-dodecyl-*N*-(3-methoxypropyl)dodecan-1-amine (**AL-A12**).

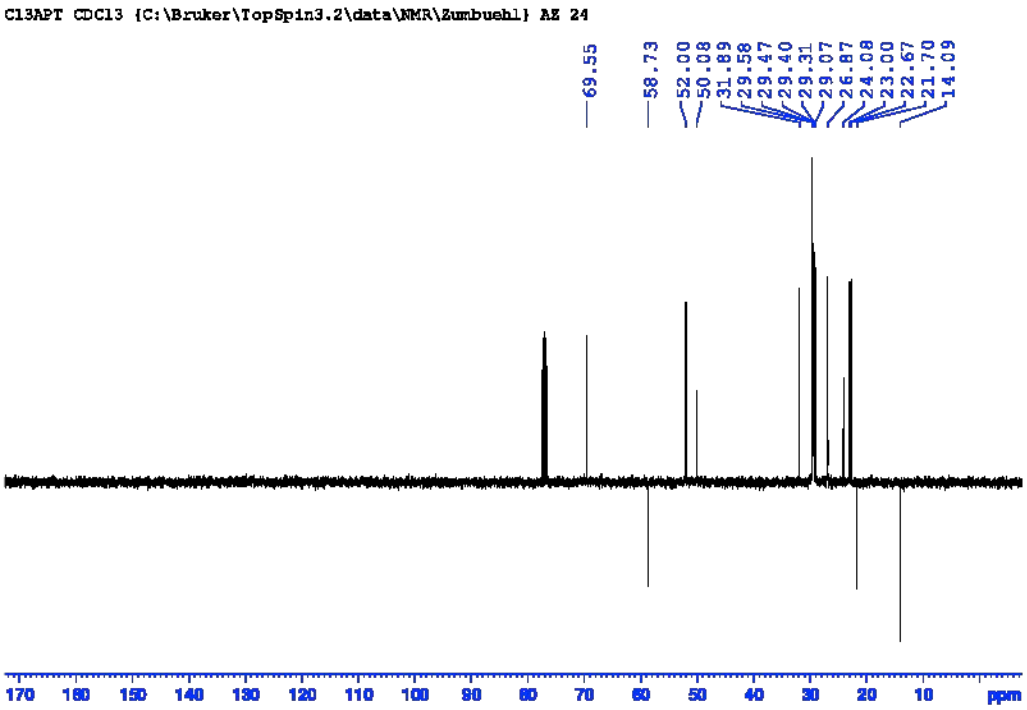

Figure S6: ^{13}C-NMR of *N*-dodecyl-*N*-(3-methoxypropyl)dodecan-1-amine (**AL-A12**).

1.3 Synthesis of 3-(didecylamino)propan-1-ol (AL-B10)

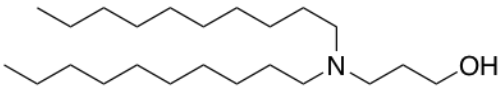

Figure S7: Chemical structure of 3-(didecylamino)propan-1-ol (**AL-B10**).

3-Amino-1-propanol (1.00 eq., 5.00 mmol, 0.38 mL) and decanal (2.20 eq., 11.00 mmol, 2.07 mL) were dissolved in DCM (25 mL) and stirred for 3 h. Sodium triacetoxyborohydride (2.20 eq., 11.00 mmol, 2.33 g) was added and the mixture was stirred for 5 days. The product was purified by column chromatography (silica; DCM/MeOH/H_2O (89:10:1 V/V/V)) to yield a slightly yellow, viscous liquid (1.380 g, 3.88 mmol, 78 %). Aldehyde is not fully reacted off.

^{1}H NMR (400 MHz, $CDCl_3$) δ = 3.75-3.72 (m, 2H), 3.14 (t, *J* =7.2 Hz, 2H), 2.97-2.93 (m, 4H), 1.92 (qui, *J* = 6.2 Hz, 2H), 1.65 (m, 4H), 1.30-1.25 (m, 28H), 0.87 (t, *J* = 6.8 Hz, 6H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ = 59.0, 51.9, 50.2, 31.8, 29.4, 29.4, 29.2, 29.1, 26.8, 22.9, 22.6, 22.0, 14.0 ppm.

IR $\tilde{\nu}$ = 2924, 2855, 1715, 1567, 1467, 1377, 1250, 1062, 1008 cm^{-1}.

HRMS (ESI$^+$) : m/z [M+H]$^+$: calculated for $[C_{23}H_{49}NO+H]^+$: 356.3892; found: 356.3880.

R_f (DCM / MeOH / H_2O, (89:10:1 V/V/V)) = 0.40.

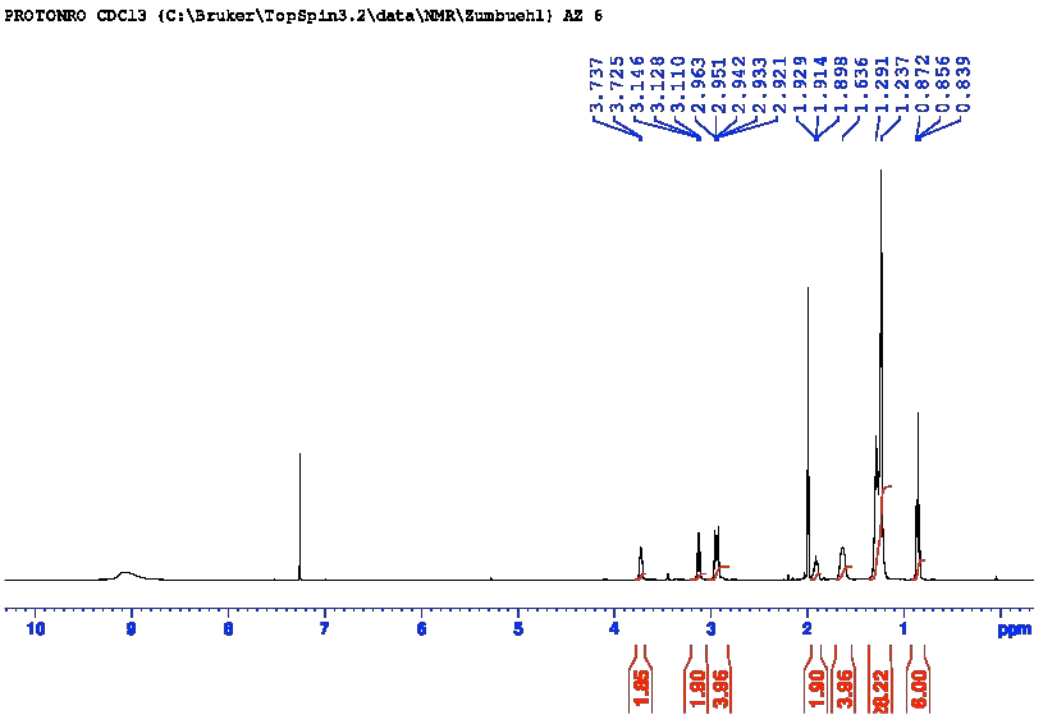

Figure S8: ^{1}H-NMR of 3-(didecylamino)propan-1-ol (**AL-B10**).

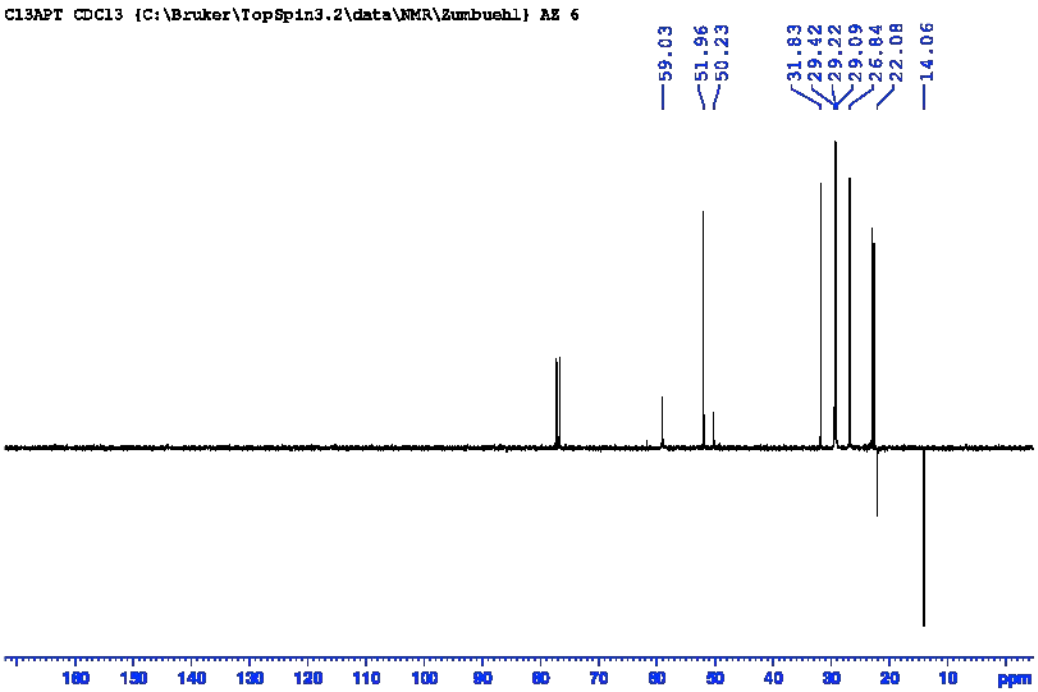

Figure S9: ^{13}C-NMR of 3-(didecylamino)propan-1-ol (**AL-B10**).

1.4 Synthesis of 3-(didodecylamino)propan-1-ol (AL-B12)

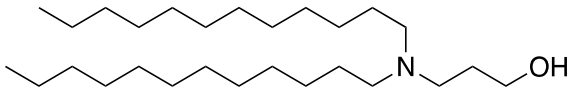

Figure S10: Chemical structure of 3-(didodecylamino)propan-1-ol (**AL-B12**).

3-Amino-1-propanol (1.00 eq., 5.00 mmol, 0.38 mL), lauraldehyde (2.20 eq, 11.00 mmol, 2.44 mL) and sodium triacetoxyborohydride (2.20 eq., 22.00 mmol, 2.33 g) were mixed in DCM and stirred for 2 days. The product was purified by column chromatography (silica, DCM/MeOH (95:5 V/V)) to yield a yellow liquid (428 mg, 1.04 mmol, 21 %). Aldehyde not fully reacted off.

^{1}H NMR (400 MHz, $CDCl_3$) δ = 3.76 (m, 2H), 3.47 (m, 1H), 3.21-3.17 (t, *J* = 7.2 Hz, 2H), 3.04-3.00 (m, 4H), 1.99-1.93 (q, *J* = 6.2 Hz, 2H), 1.68 (m, 4H), 1.30-1.24 (m, 36H), 0.88-0.85 (t, *J* = 6.7 Hz, 6H) ppm.

^{13}C NMR (100MHz, $CDCl_3$) δ = 61.4, 59.0, 52.0, 51.9, 31.8, 29.5, 29.3, 29.3, 29.0, 26.7, 26.3, 22.8, 22.6, 21.1, 14.0 ppm.

IR ν̃ = 2924, 2855, 1713, 1379, 1237, 1008 cm^{-1}.

HRMS (ESI$^+$) : m/z [M+H]$^+$: calculated for $[C_{27}H_{57}NO + H]^+$: 412.4513; found : 412.4512

R_f (DCM/MeOH (95:5 V/V)): 0.67.

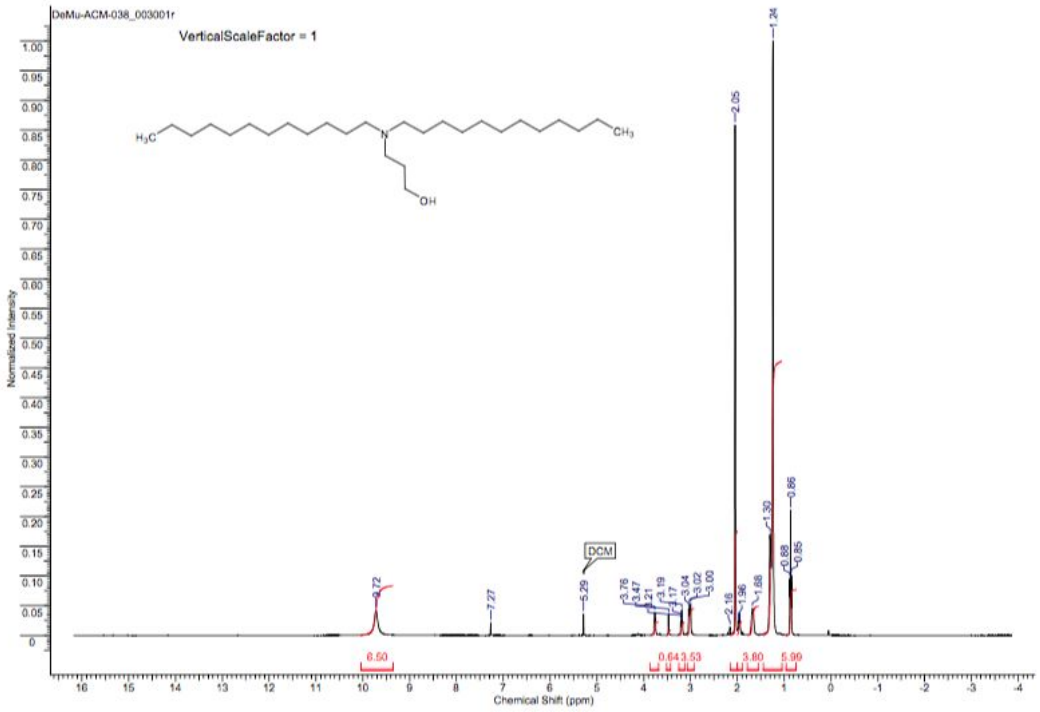

Figure S11: ^{1}H-NMR of 3-(didodecylamino)propan-1-ol (**AL-B12**).

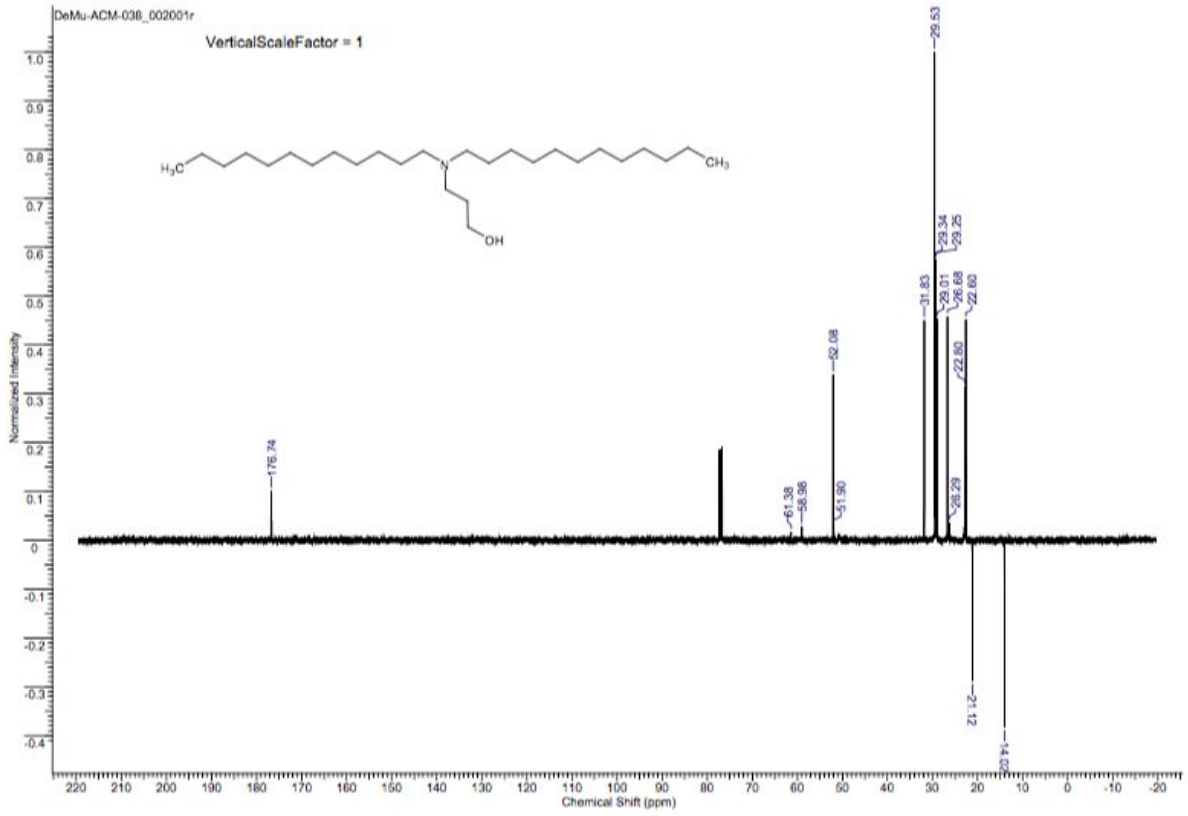

Figure S12: ^{13}C-NMR of 3-(didodecylamino)propan-1-ol (**AL-B12**).

1.5 Synthesis of 3-(didecylamino)propane-1,2-diol (**AL-C10**)

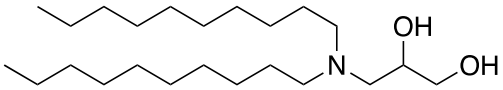

Figure S13: Chemical structure of 3-(didecylamino)propane-1,2-diol (**AL-C10**).

(±)-3-Amino-1,2-propanediol (1.00 eq., 5.00 mmol, 0.51 mL), decanal (2.20 eq, 11.00 mmol, 2.07 mL) and sodium triacetoxyborohydride (2.20 eq., 11.00 mmol, 2.33 g) were mixed in DCM and stirred for 2 days. The product was purified by column chromatography (silica; EtOAc/MeOH (98:2 V/V) → EtOAc/MeOH (90:10 V/V)) to yield a yellow liquid (773 mg, 2.081 mmol, 42 %).

NMR data still showed impurities after several purification steps.

HRMS (ESI$^+$) : m/z [M+H]$^+$: calculated for $[C_{23}H_{49}NO_2 + H]^+$: 372.3836; found : 372.3831

R_f (EtOAc/MeOH (90:10 V/V)): 0.25.

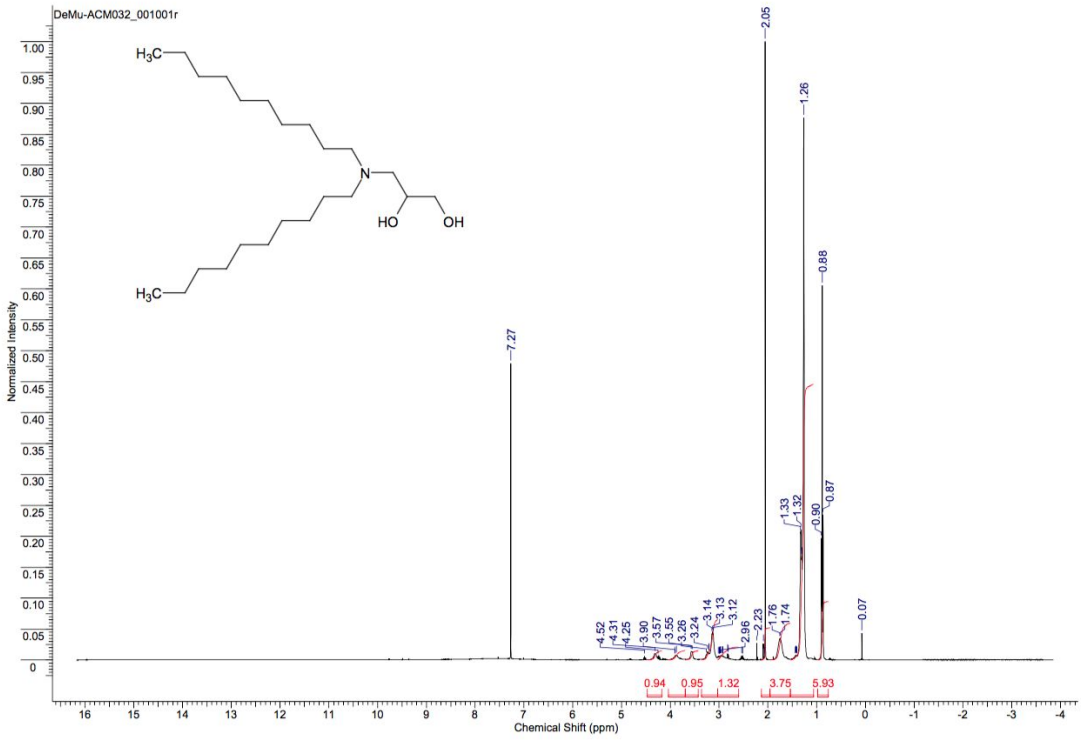

Figure S14: ^{1}H-NMR of 3-(didecylamino)propane-1,2-diol (**AL-C10**).

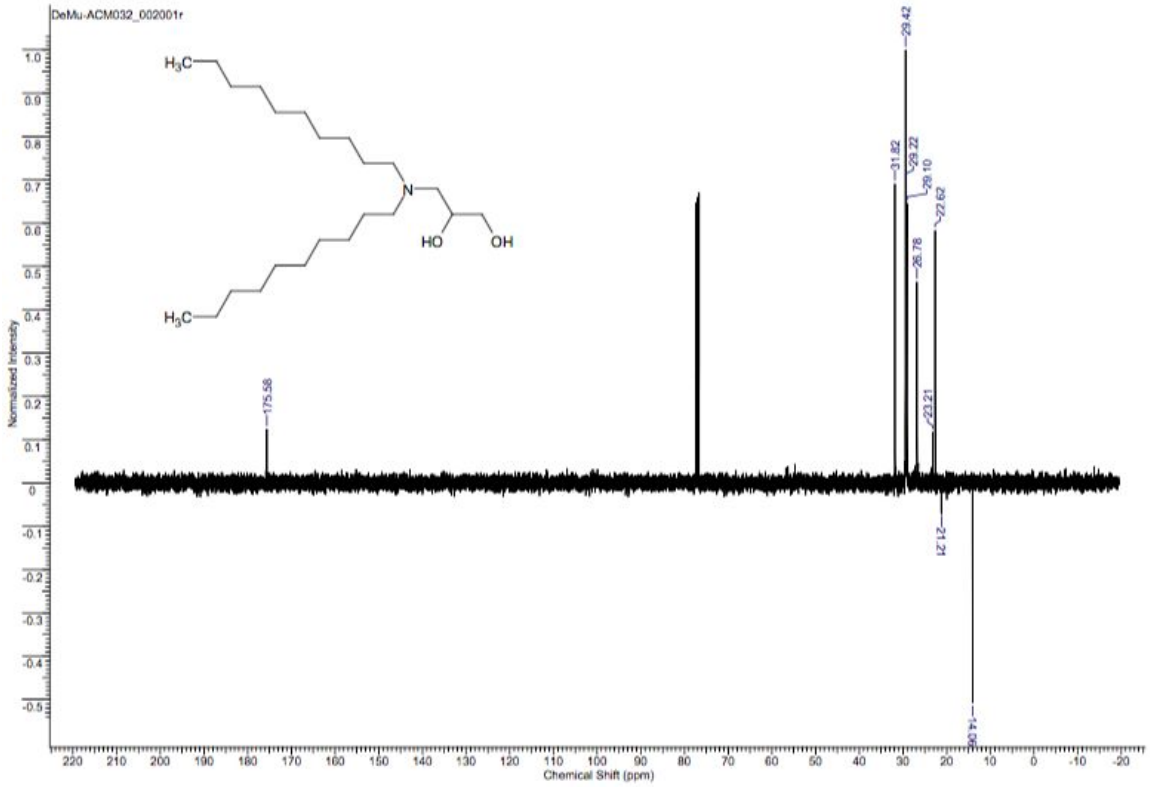

Figure S15: ^{13}C-NMR of 3-(didecylamino)propane-1,2-diol (**AL-C10**).

1.6 Synthesis 3-(didodecylamino)-1,2-propanediol (AL-C12)

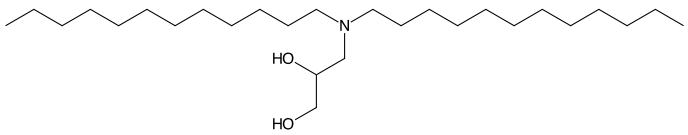

Figure S16: Chemical structure of 3-(didodecylamino)-1,2-propanediol (**AL-C12**).

(±)-3-Amino-1,2-propanediol (1.00 eq., 5.00 mmol, 0.51 mL), lauraldehyde (2.20 eq, 11.00 mmol, 2.44 mL) and sodium triacetoxyborohydride (2.20 eq., 11.00 mmol, 2.33 g) were mixed in DCM and stirred for 2 days. The product was purified by column chromatography (silica; DCM/MeOH (98:2 V/V)). The yield was too low to be determined, but still sufficient for characterization by NMR and HRMS.

^{1}H NMR (600 MHz, $CDCl_3$) δ = 4.17-4.11 (m, 1H), 3.73-3.67 (m, 1H), 3.62-3.56 (m, 1H), 3.18-2.93 (m, 5H), 1.75-1.57 (m, 2H),1.36-1.20 (m, 38H), 0.91-0.85 (m, 6H) ppm.

^{13}C NMR (150 MHz, CDCl3) δ = 66.8, 64.7, 57.3, 54.2, 32.1, 29.8, 29.8, 29.7, 29.6, 29.5, 29.4, 27.1, 23.8, 22.9, 14.3 ppm.

HRMS (ESI$^+$): m/z [M+H]$^+$: calculated for $[C_{27}H_{57}NO_2 + H]^+$: 428.4462; found: 428.4462.

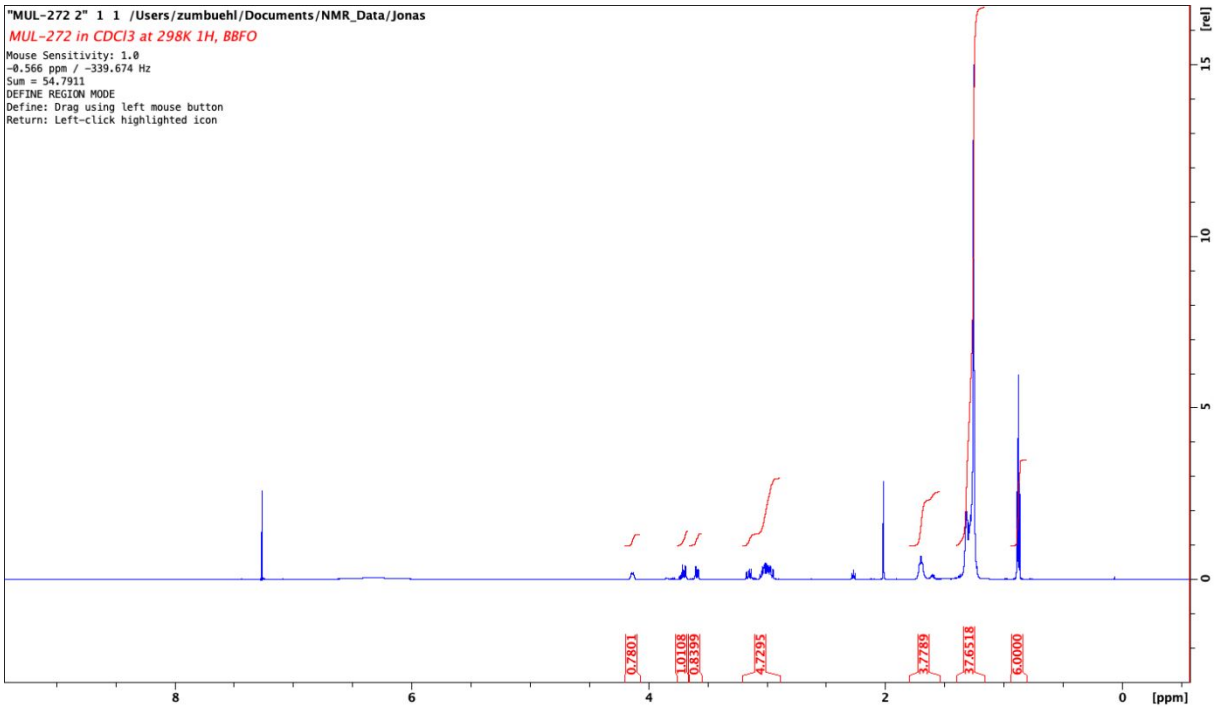

Figure S17: ^{1}H-NMR of 3-(Didodecylamino)-1,2-propanediol (**AL-C12**).

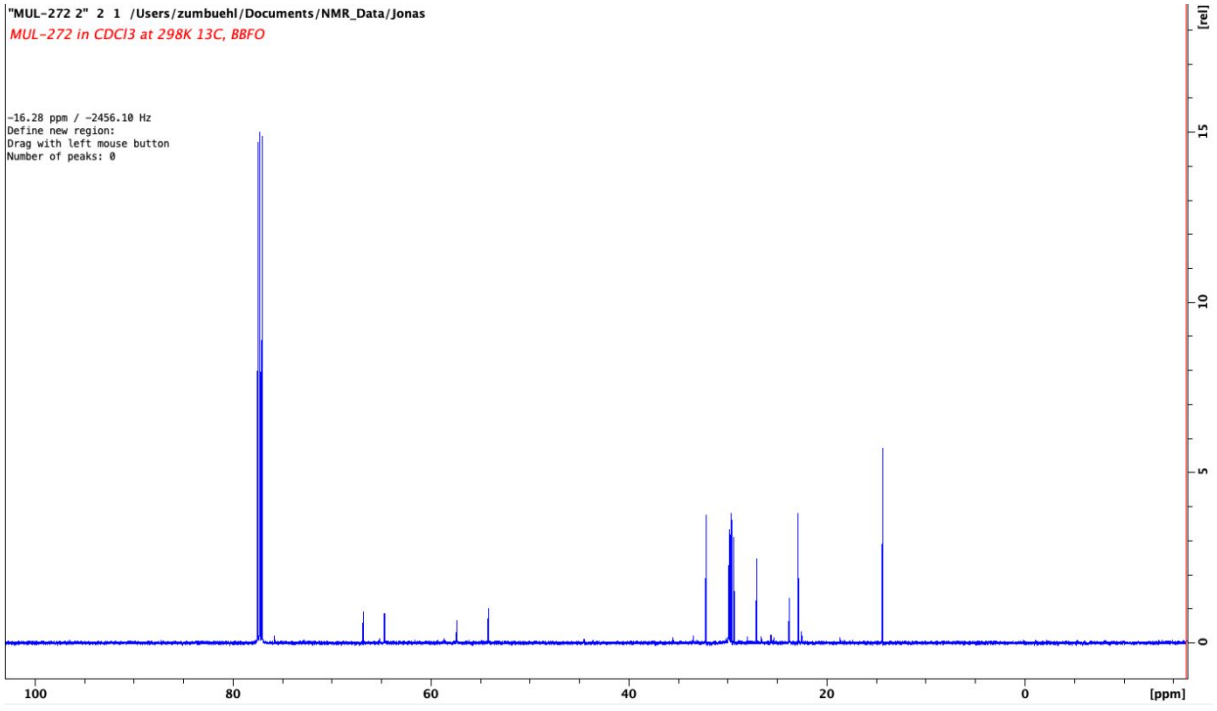

Figure S18: ^{13}C-NMR of 3-(Didodecylamino)-1,2-propanediol (**AL-C12**).

1.7 Synthesis of *N*,*N*-didecyl-*N*,*N*-dimethylethane-1,2-diamine (**AL-D10**)

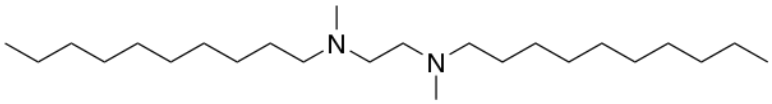

Figure S19: Chemical structure of *N*,*N*'-didecyl-*N*,*N*'-dimethylethane-1,2-diamine (**AL-D10**).

N,*N*''-Dimethylethylenediamine (1.00 eq., 5.00 mmol, 0.54 mL), and decanal (2.20 eq., 11.00 mmol, 2.07 mL) were dissolved in DCM (25 mL) and stirred for 3 h. Sodium triacetoxyborohydride (2.20 eq., 11.00 mmol, 2.33 g) was added and the reaction was stirred for 2 days. The product was purified by column chromatography (silica; DCM/MeOH/H_2O (89:10:1 V/V/V)) to yield a slightly yellow, cloudy and viscous liquid (1.924 g, 5.21 mmol). Aldehyde not fully reacted off.

^{1}H NMR (400 MHz, $CDCl_3$) δ = 3.04 (s, 4H), 2.75-2.71 (m, 4H), 2.53 (s, 6H), 1.61-1.57 (m, 4H), 1.28-1.25 (m, 28H), 0.87 (t, *J* = 6.9 Hz, 12 H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ = 56.8, 51.6, 40.6, 31.8, 29.4, 29.2, 27.0, 24.8, 22.6, 21.8, 14.0 ppm.

IR $\tilde{\nu}$ = 2924, 2854, 1713, 1568, 1466, 1406, 1377, 1257 cm^{-1}.

HRMS (ESI$^+$) : m/z [M+H]$^+$: calculated for $[C_{24}H_{52}N_2+H]^+$: 369.4209; found: 369.4209.

R_f (DCM / MeOH / H_2O, (89:10:1 V/V/V)) = 0.39.

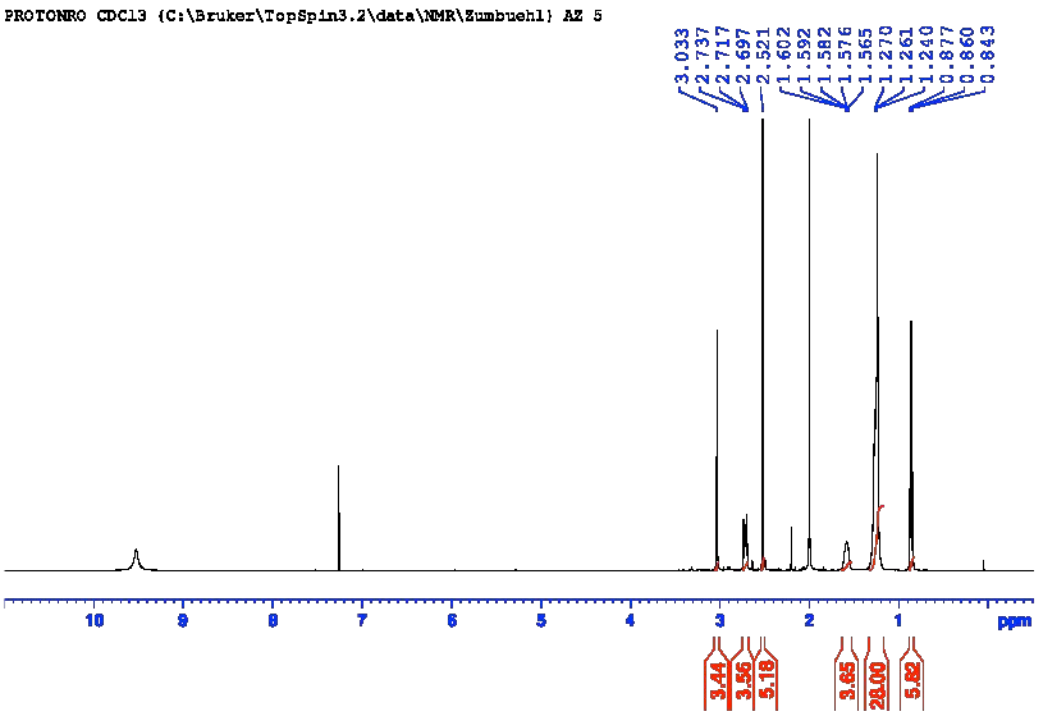

Figure S20: ^{1}H-NMR of *N,N*'-didecyl-*N,N*'-dimethylethane-1,2-diamine (**AL-D10**).

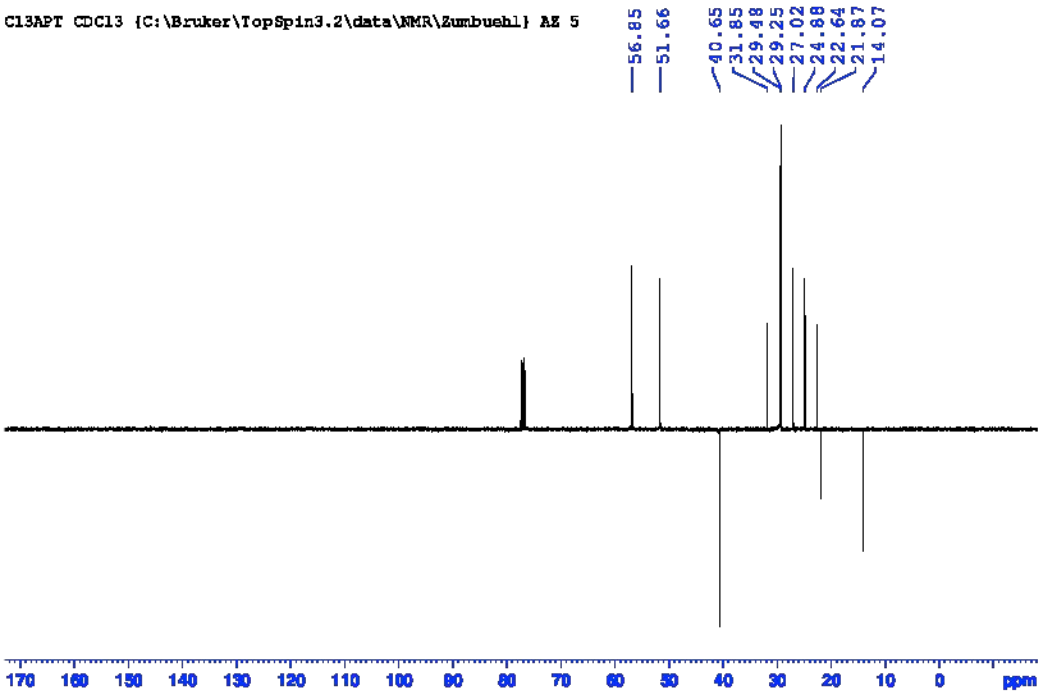

Figure S21: ^{13}C-NMR of *N,N*'-didecyl-*N,N*'-dimethylethane-1,2-diamine (**AL-D10**).

1.8 Synthesis of *N,N,N',N'*-Tetrakis(decyl)propane-1,3-diamine (**AL-E10**)

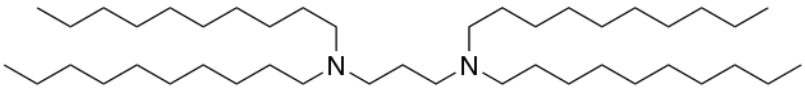

Figure S22: Chemical structure of *N,N,N',N'*-Tetrakis(decyl)propane-1,3-diamine (**AL-E10**).

1,3-Diaminopropane (1.00 eq., 5.00 mmol, 0.42 mL), and decanal (4.40 eq., 22.00 mmol, 4.14 mL) were dissolved in DCM (25 mL) and stirred for 3 h. Sodium triacetoxyborohydride (4.40 eq., 22.00 mmol, 4.66 g) was added and the reaction was stirred for 2 days. The product was purified by column chromatography (silica; EtOAc/MeOH (98:2) → EtOAc/MeOH (90:10)) to yield a slightly yellow liquid (855 mg, 1.36 mmol, 27 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 3.10 (m, 4H), 2.95-2.91 (m, 8H), 2.43-2.39 (m, 2H), 1.77-1.66 (m, 8H), 1.33-1.27 (m, 56H), 0.89 (t *J* = 7.0 Hz, 12H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ = 52.5, 51.0, 31.8, 29.4, 29.4, 29.2, 29.1, 26.9, 23.4, 22.6, 14.1 ppm.

IR $\tilde{\nu}$ = 2922, 2853, 1715, 1571, 1466, 1377, 1259 cm^{-1}.

HRMS (ESI$^+$): m/z [M+H]$^+$: calculated for $[C_{43}H_{90}N_2+H]^+$: 635.7182; found: 635.7178.

R_f (EtOAc / MeOH (9:1 V/V)) = 0.54.

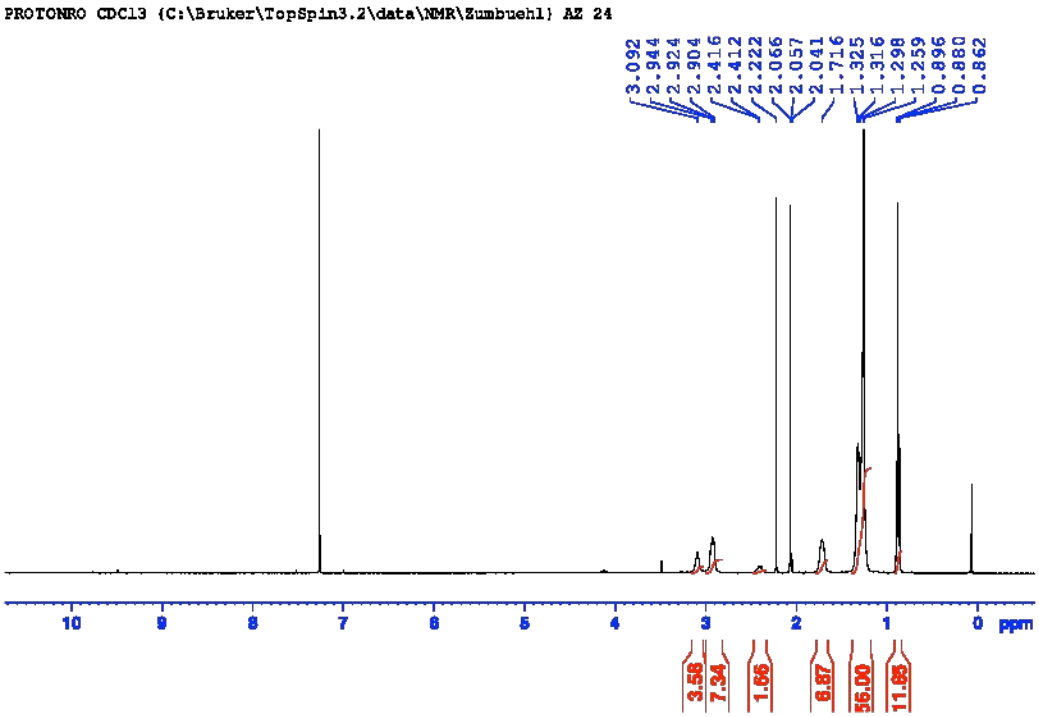

Figure S23: [1]H-NMR of *N,N,N',N'*-Tetrakis(decyl)propane-1,3-diamine (**AL-E10**).

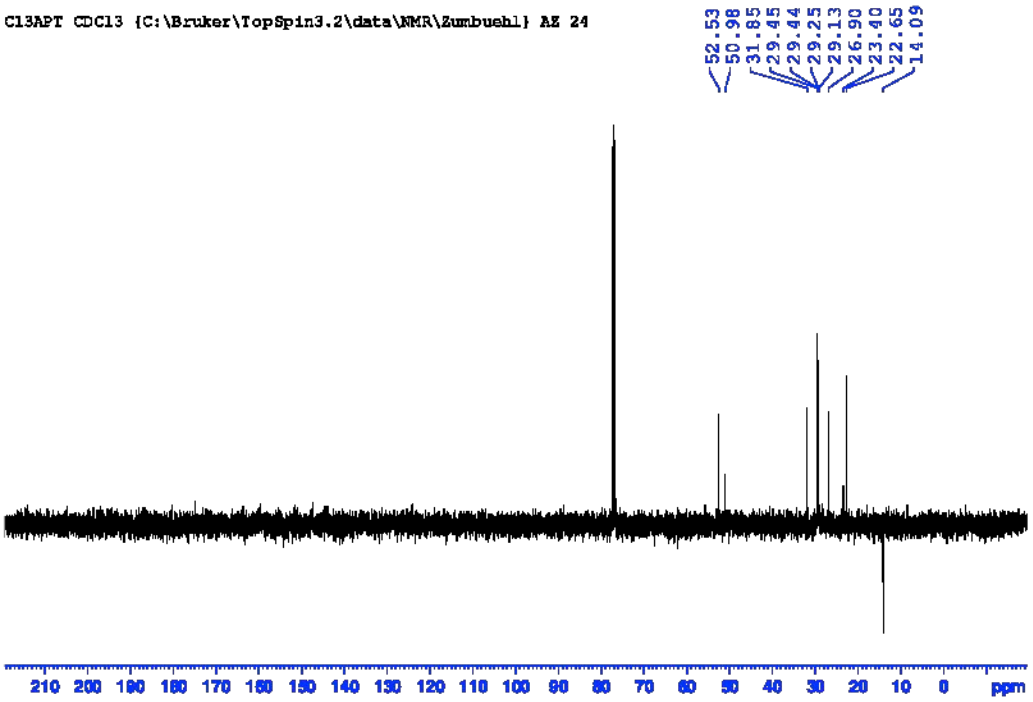

Figure S24: [13]C-NMR of *N,N,N',N'*-Tetrakis(decyl)propane-1,3-diamine (**AL-E10**).

1.9 Synthesis of *N,N,N',N'*-tetradodecylpropane-1,3-diamine (**AL-E12**)

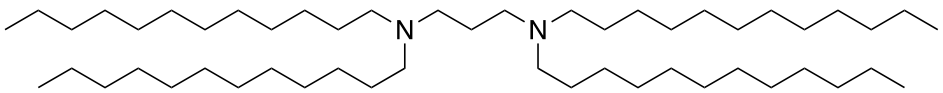

Figure S25: Chemical structure of *N,N,N',N'*-tetradodecylpropane-1,3-diamine (**AL-E12**).

1,3-Diaminopropane (1.00 eq., 5.00 mmol, 0.42 mL), lauraldehyde (4.40 eq, 22.00 mmol, 4.88 mL) and sodium triacetoxyborohydride (4.40 eq., 22.00 mmol, 4.66 g) were mixed in DCM and stirred for 5 days. The product was purified by column chromatography (silica; DCM/MeOH (98:2 V/V)) to yield a yellow liquid (26 mg, 0.04 mmol, <1 %). Aldehyde not fully reacted off.

^{1}H NMR (400 MHz, $CDCl_3$) δ = 3.10-3.06 (t, *J* = 7.8 Hz 4H), 2.96-2.92 (q, *J* = 4.2 Hz, 8H), 2.24-2.08 (m, 2H), 1.61 (m, 8H), 1.29-1.25 (m, 72H), 0.89-0.85 (t, *J* = 6.9 Hz, 12H) ppm.

^{13}C NMR (100 MHz, CDCl3) δ = 51.8, 51.6, 49.8, 31.9, 29.6, 29.3, 29.1, 26.8, 22.9, 20.0, 22.6, 21.7, 14.0 ppm.

IR ν̃ = 2923, 2854, 1714, 1565, 1467, 1361, 1252, 1008 cm^{-1}.

HRMS (ESI$^+$): m/z [M+H]$^+$: calculated for $[C_{51}H_{106}N_2 + H]^+$: 747.8429; found : 747.8426

R_f (DCM/MeOH (98:2 V/V)): 0.16.

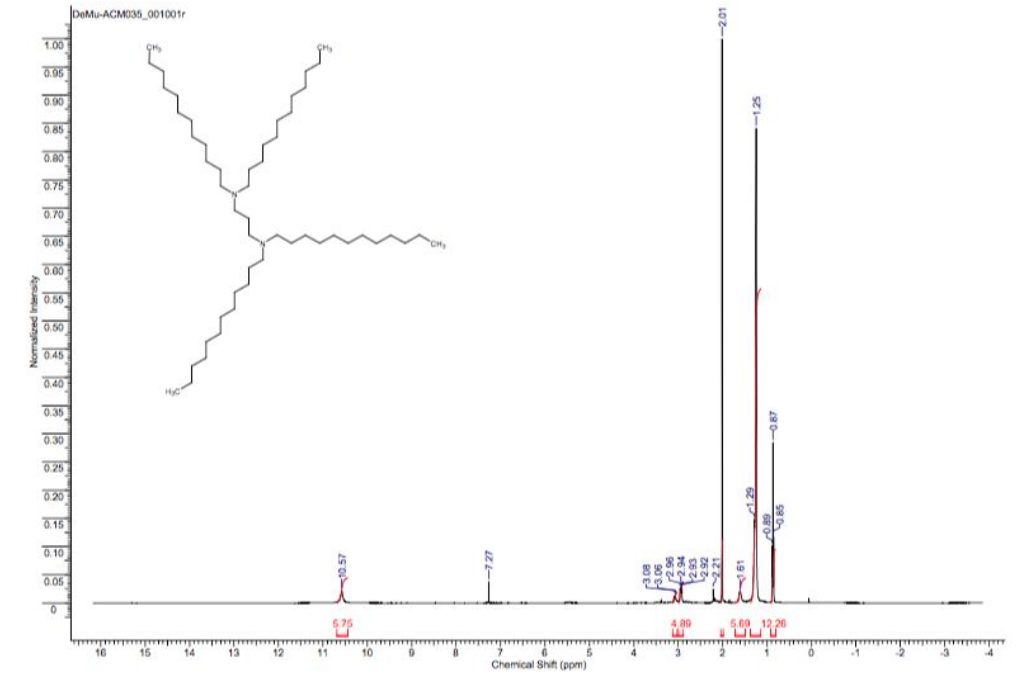

Figure S26: ^{1}H-NMR of *N,N,N',N'*-tetradodecylpropane-1,3-diamine (**AL-E12**).

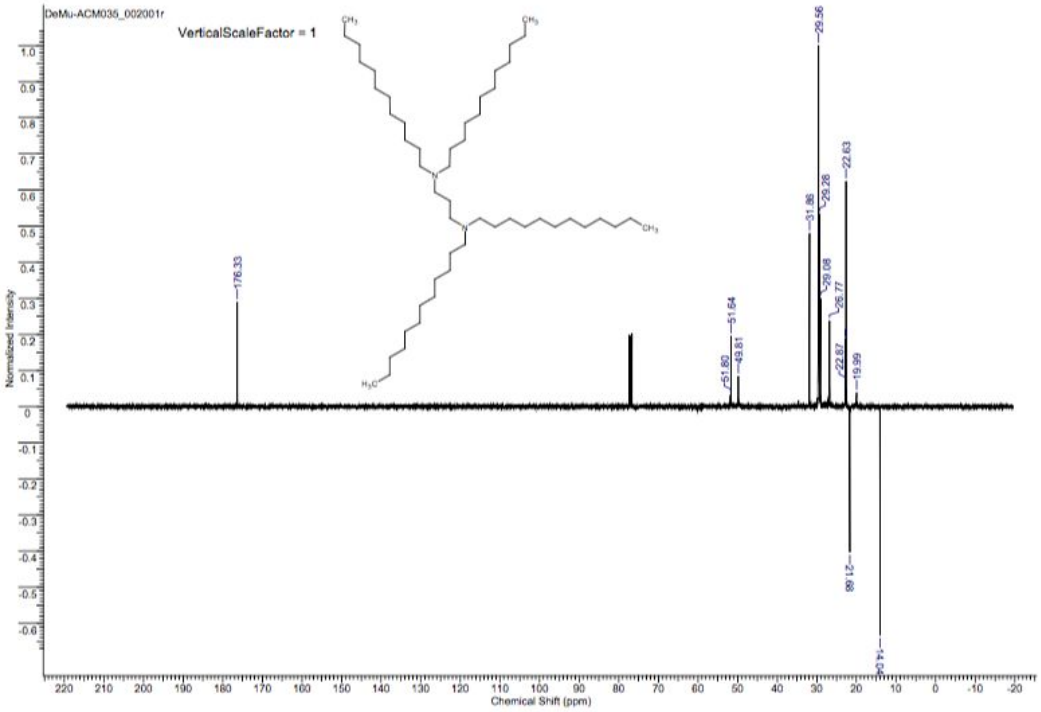

Figure S27: ^{13}C-NMR of *N,N,N',N'*-tetradodecylpropane-1,3-diamine (**AL-E12**).

1.10 Synthesis of (Benzyl)didecylamine (AL-F10)

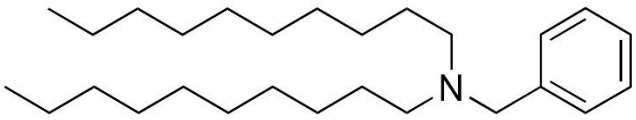

Figure S28: Chemical structure of (Benzyl)didecylamine (**AL-F10**).

Benzylamine (1.00 eq., 5.00 mmol, 0.55 mL), and decanal (2.20 eq., 11.00 mmol, 2.07 mL) were dissolved in DCM (25 mL) and stirred for 3 h. Sodium triacetoxyborohydride (2.20 eq., 11.00 mmol, 2.33 g) was added and the mixture was stirred for 2 days. The product was purified by column chromatography (Silica; EtOAc/MeOH (98:2 V/V) → EtOAc/MeOH (90:10 V/V)) to yield a dark yellow liquid (666 mg, 1.72 mmol, 34.4 %).

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.27 – 7.10 (m, 5H), 3.46 (s, 2H), 2.36 – 2.26 (m, 4H), 1.45 – 1.31 (m, 4H), 1.17 (s, 28H), 0.81 (t, *J* = 6.8 Hz, 6H) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$): δ = 140.5, 129.0, 128.2, 126.7, 58.8, 54.0, 32.1, 29.83, 29.77, 29.75, 29.5, 27.5, 27.2, 22.8, 14.3 ppm.

HRMS (ESI$^+$): m/z [M+H]$^+$: calculated for $[C_{27}H_{49}N + H]^+$: 388.3938; found : 388.3933.

R_f(DCM:MeOH (92:8 V/V)): 0.94.

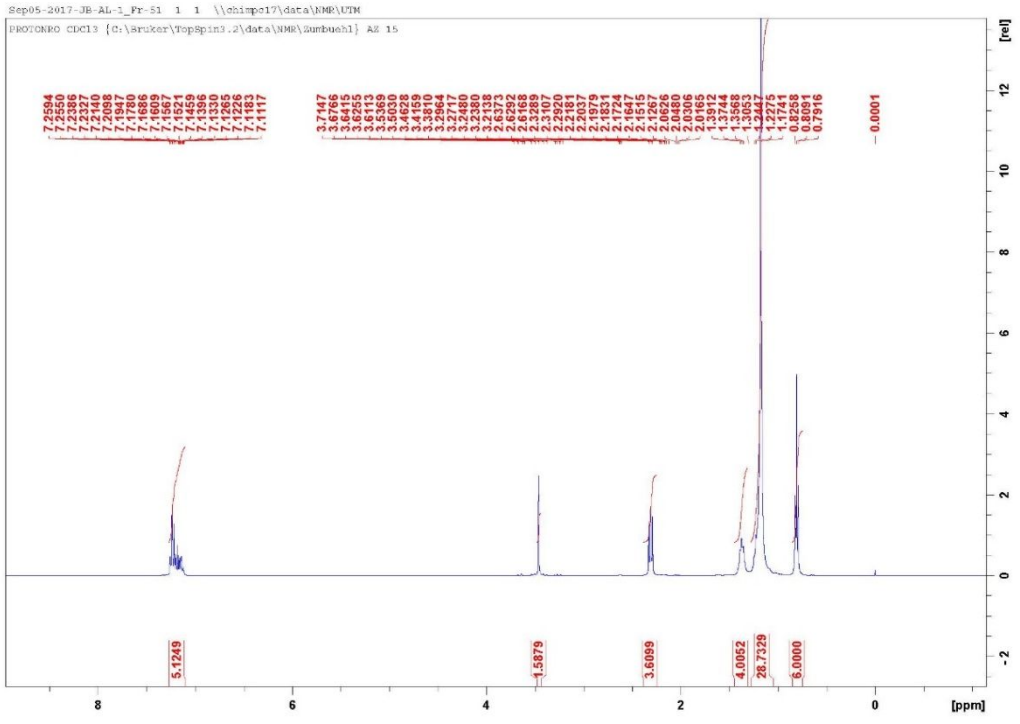

Figure S29: ^{1}H-NMR of (Benzyl)didecylamine (**AL-F10**).

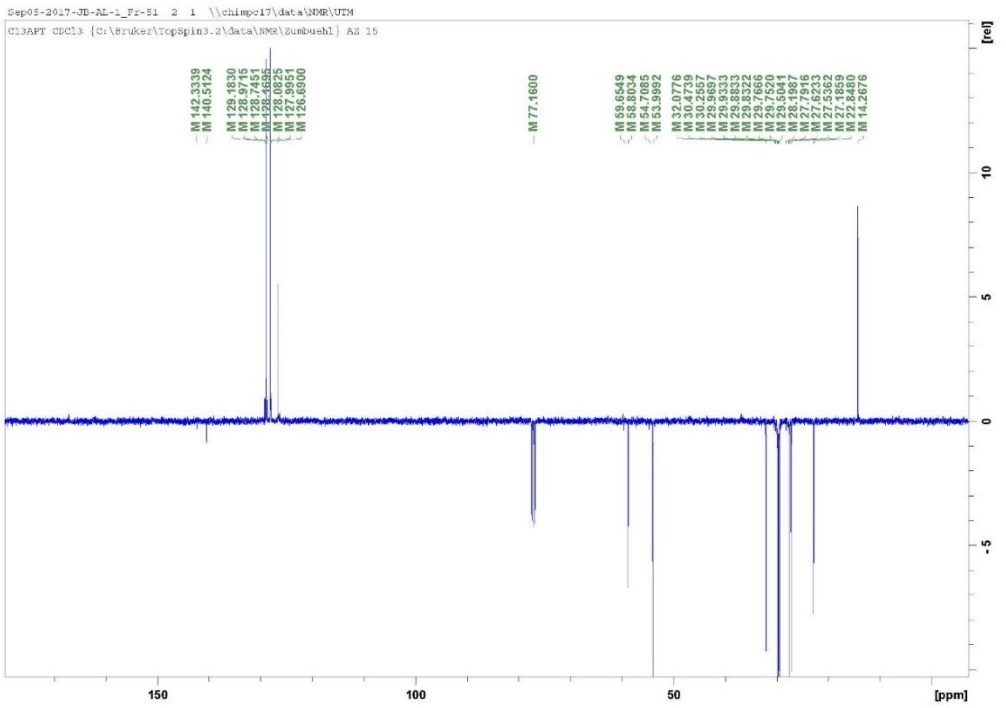

Figure S30: ^{13}C-NMR of (Benzyl)didecylamine (**AL-F10**).

1.11 Synthesis of [(1*H*-Imidazol-2-yl)methyl]didecylamine (AL-G10)

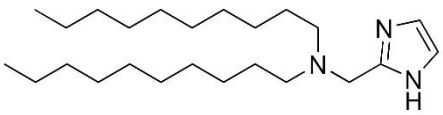

Figure S31: Chemical structure of [(1*H*-Imidazol-2-yl)methyl]didecylamine (**AL-G10**).

2-(Aminomethyl)imidazole dihydrochloride (1.00 eq., 5.00 mmol, 0.85 g), and decanal (2.20 eq., 11.00 mmol, 2.07 mL) were dissolved in DCM (25 mL) and stirred for 3 h. Sodium triacetoxyborohydride (2.20 eq., 11.00 mmol, 2.33 g) was added and the mixture was stirred for 2 days. The product was purified by column chromatography (Silica; EtOAc/MeOH (98:2 V/V) → EtOAc/MeOH (90:10 V/V)) to yield a yellow liquid (1.36 g, 3.59 mmol, 71.9 %).

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 11.23 (br s, 2H), 7.01 – 6.93 (m, 2H), 5.22 (s, 1H), 4.39 (s, 1H), 3.58 – 3.55 (m, 1 H), 2.86 – 2.81 (m, 2H), 1.98 (br s, 4H), 1.58 – 1.46 (m, 3H), 1.18 (br s, 28H), 0.83 – 0.79 (m, 6H) ppm.

The integrals are not fitting well due to impurities which could not be removed by chromatography. Residual solvent peaks were found for DCM (5.33 ppm) and EtOAc 1.26 (s), 2.05 (t), 4.12 (q)

^{13}C-NMR (100 MHz, $CDCl_3$): δ = 138.1, 122.9, 63.1, 52.9, 49.5, 37.7, 34.6, 32.9, 32.0, 32.0, 29.7, 29.7, 29.6, 29.6, 29.6, 29.5, 29.4, 29.3, 29.2, 29.0, 27.3, 27.3, 26.9, 25.9, 23.4, 22.8, 22.2, 14.2 ppm.

HRMS (ESI$^+$): m/z [M+H]$^+$: calculated for $[C_{24}H_{47}N_3 + H]^+$: 378.3843; found : 378.3843.

R_f(DCM:MeOH (92:8 V/V)): 0.64.

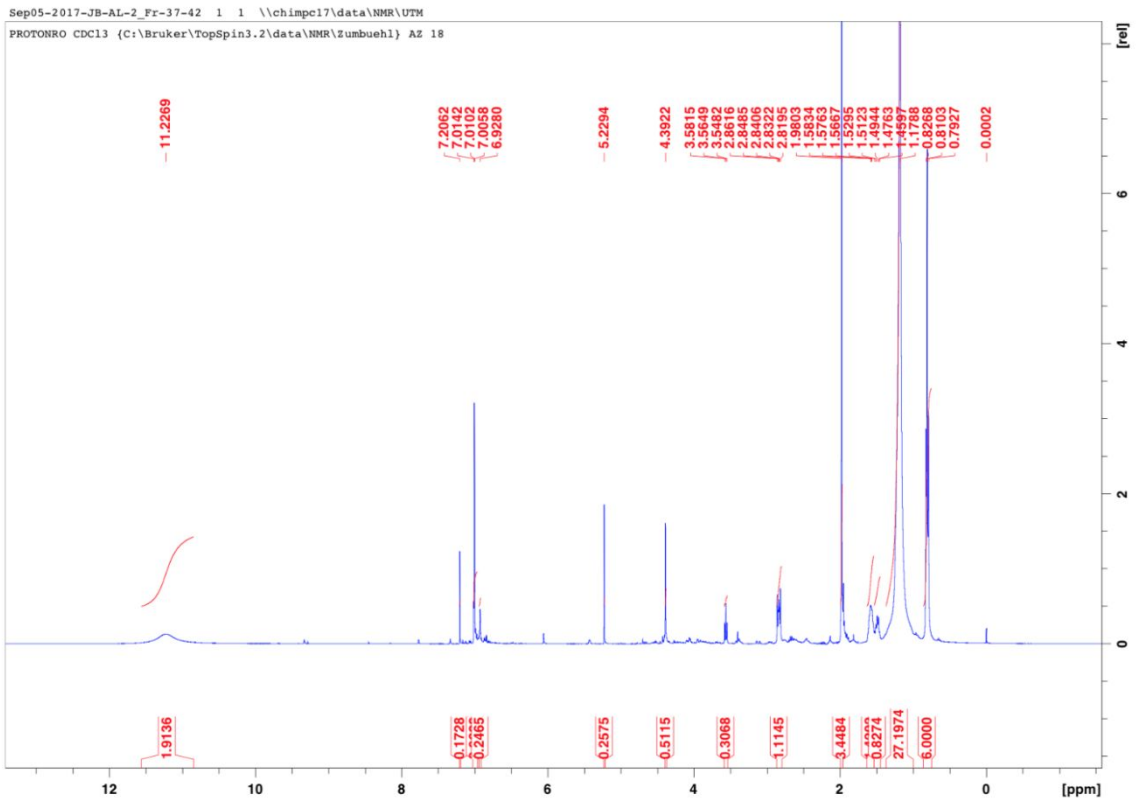

Figure S32: ^{1}H-NMR of [(1*H*-Imidazol-2-yl)methyl]didecylamine (**AL-G10**).

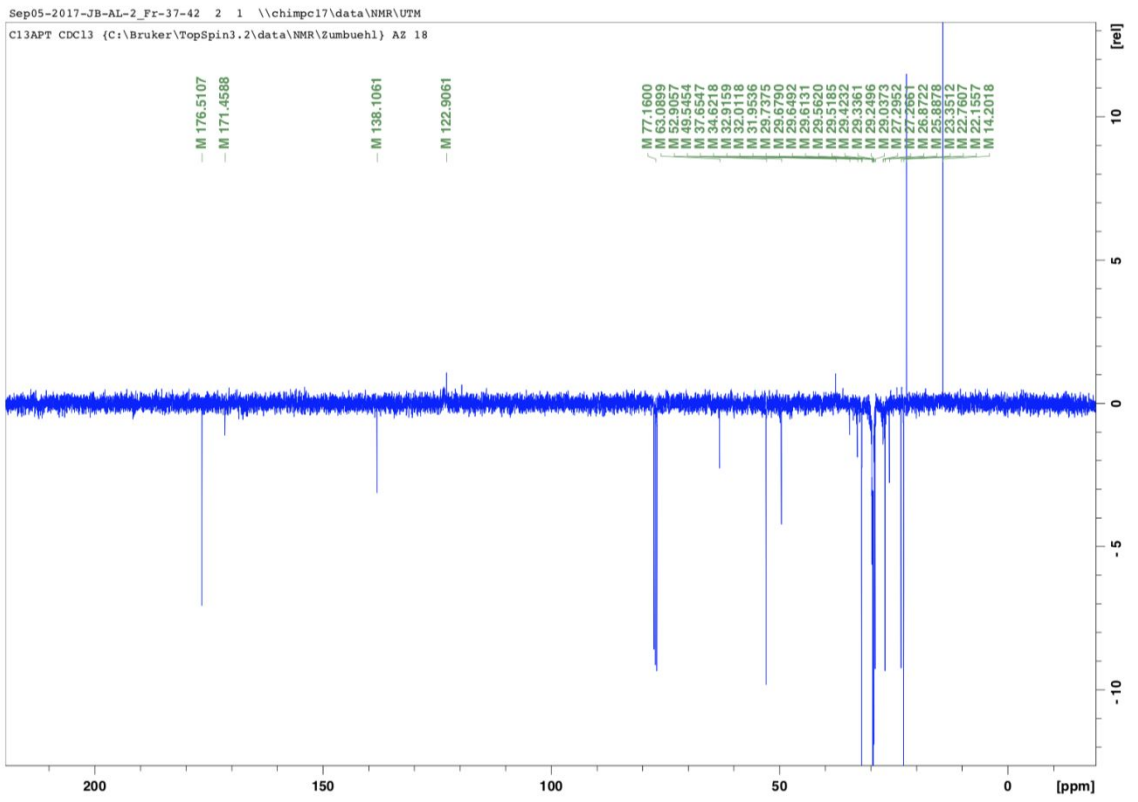

Figure S33: ^{13}C-NMR of [(1*H*-Imidazol-2-yl)methyl]didecylamine (**AL-G10**).

1.12 Synthesis [(4-Pyridyl)methyl]didecylamine (AL-H10)

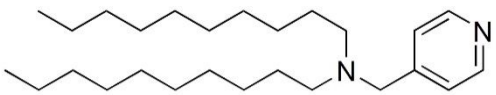

Figure S34: Chemical structure of [(4-Pyridyl)methyl]didecylamine (**AL-H10**).

4-(Aminomethyl)pyridine (1.00 eq., 5.00 mmol, 0.51 mL), and decanal (2.20 eq., 11.00 mmol, 2.07 mL) were dissolved in DCM (25 mL) and stirred for 3 h. Sodium triacetoxyborohydride (2.20 eq., 11.00 mmol, 2.33 g) was added and the mixture was stirred for 2 days. The product was purified by column chromatography (Silica; EtOAc/MeOH (98:2 V/V) → EtOAc/MeOH (90:10 V/V)) to yield a brown liquid (1.75 g, 4.51 mmol, 90.1 %). The spectrum still shows small acetic acid contaminations at approx. 2.1 ppm (^{1}H-NMR), 175 ppm, and 21 ppm (both ^{13}C-NMR) that could not be removed with column chromatography.

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.54 – 8.49 (m, 2H), 7.32 – 7.29 (m, 2H), 3.56 (s, 2H), 2.44 – 2.36 (m, 4H), 1.49 – 1.39 (m, 4H), 1.24 (s, 28H), 0.87 (t, *J* = 6.8 Hz, 6H) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$): δ = 150.3, 149.1, 124.0, 57.8, 54.2, 32.0, 29.8, 29.71, 29.66, 29.5, 27.5, 27.0, 22.8, 14.2 ppm.

HRMS (ESI$^+$): m/z [M+H]$^+$: calculated for $[C_{26}H_{48}N_2 + H]^+$: 389.3890; found : 389.3890.

R_f(DCM:MeOH (92:8 V/V)): 0.78.

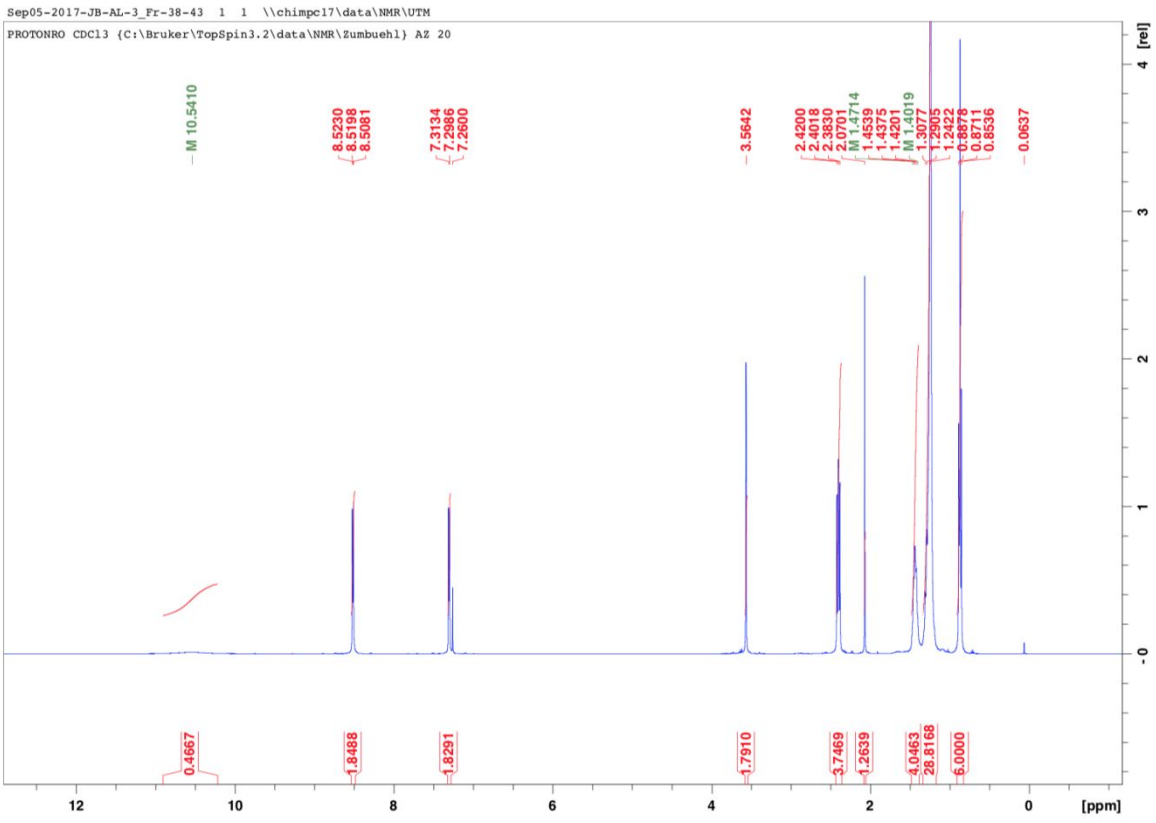

Figure S35: ^{1}H-NMR of [(4-Pyridyl)methyl]didecylamine (**AL-H10**).

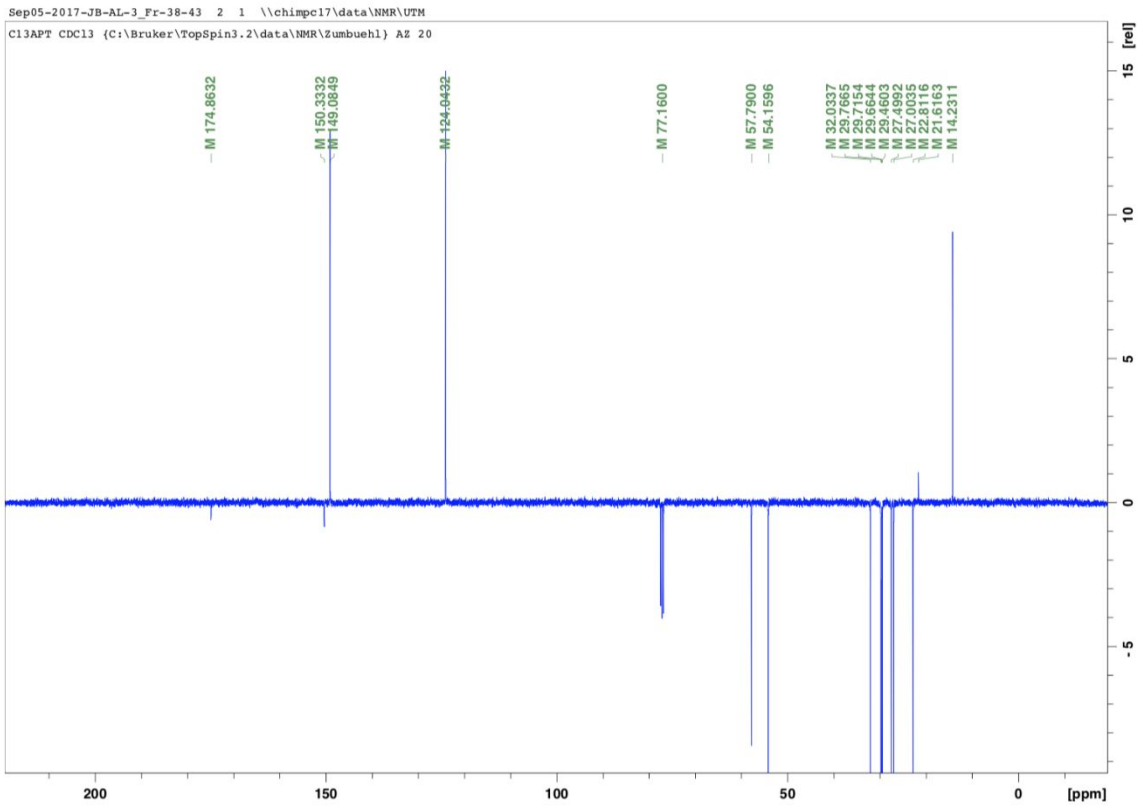

Figure S36: ^{13}C-NMR of [(4-Pyridyl)methyl]didecylamine (**AL-H10**).

2. *In vitro* experiments

2.1 Transfection experiments

Table S1: Results of transfection experiments for aminolipid- and DOTAP:chol-based lipoplexes.

Substance	Setting	GFP positive cells [%]	MFI [RFU]
AL-A10	1 µg DNA, 1:1 ratio	7.7	801
	2 µg DNA, 1:1 ratio	28.7	1033
AL-A12	1 µg DNA, 1:1 ratio	10.3	785
	2 µg DNA, 1:1 ratio	31.5	1606
AL-B10	1 µg DNA, 1:1 ratio	5.0	384
	2 µg DNA, 1:1 ratio	11.5	928
AL-B12	1 µg DNA, 1:1 ratio	8.6	469
	2 µg DNA, 1:1 ratio	8.3	358
AL-C10	1 µg DNA, 1:1 ratio	8.3	487
	2 µg DNA, 1:1 ratio	9.5	215
AL-C12	1 µg DNA, 1:1 ratio	6.0	658
	2 µg DNA, 1:1 ratio	4.2	451
AL-D10	1 µg DNA, 1:1 ratio	7.9	354
	2 µg DNA, 1:1 ratio	11.6	224
AL-E10	1 µg DNA, 1:1 ratio	4.7	577
	2 µg DNA, 1:1 ratio	11.5	928

AL-F10	1 µg DNA, 1:1 ratio	4.5	906
AL-G10	1 µg DNA, 1:1 ratio	4.7	714
AL-H10	1 µg DNA, 1:1 ratio	2.7	764
DOTAP:chol	1 µg DNA, 1:1 ratio	5.7	507
	2 µg DNA, 1:1 ratio	13.2	756
Lipofectamine 3000	1 µg DNA, 1:1 ratio	47.5	7632

2.2 Cytotoxicity experiments

Table S2: LC10, LC50, and LC90 values corresponding to 90%, 50%, and 10% cell viability for aminolipid- and DOTAP:chol-based systems. The values were calculated from MTT-assay data using the non-linear dose-response function in Origin 2018.

Substance	LC10 (90% viability) [µM]	LC50 (50% viability) [µM]	LC90 (10% viability) [µM]
AL-A10	3.7	16.8	76.5
AL-A12	4.2	43.9	456.9
AL-B10	5.4	9.3	15.9
AL-B12	4.1	9.3	21.1
AL-C10	2.2	4.8	10.4
AL-C12	0.8	9.7	120.3
AL-D10	0.9	3.0	9.5
AL-E10	1.7	20.6	251.9
AL-F10	2.2	75.3	2548.8
AL-G10	9.7	42.2	182.7
AL-H10	2.0	20.6	213.7
DOTAP:chol	2.4	24.4	254.6

Table S3: Average cell viability results of three MTT assays.

Substance	% Survival (4 µM)	% Survival (16 µM)	% Survival (25 µM)	% Survival (64 µM)	% Survival (128 µM)
AL-A10	77.5 ± 0.7	72.9 ± 1.0	65.3 ± 0.4	7.2 ± 0.3	2.5 ± 0.4
AL-A12	96.3 ± 0.7	88.9 ± 1.9	78.2 ± 2.8	48.2 ± 0.6	33.6 ± 0.5
AL-B10	79.2 ± 0.9	69.2 ± 0.6	3.4 ± 0.0	1.7 ± 0.2	1.7 ± 0.2
AL-B12	80.2 ± 1.8	67.4 ± 0.7	13.4 ± 0.2	2.0 ± 0.2	3.1 ± 1.9
AL-C10	62.8 ± 1.1	2.9 ± 0.1	1.4 ± 0.0	2.0 ± 0.6	2.2 ± 0.7
AL-C12	58.3 ± 0.2	53.6 ± 0.6	34.7 ± 0.3	3.6 ± 1.8	3.6 ± 0.1
AL-D10	36.5 ± 1.1	2.4 ± 0.1	3.3 ± 0.4	3.4 ± 0.1	3.5 ± 0.2
AL-E10	71.8 ± 1.5	62.2 ± 0.9	50.7 ± 0.9	22.5 ± 1.6	12.3 ± 1.2
AL-F10	84.7 ± 4.5	68.8 ± 2.0	69.4 ± 1.0	54.1 ± 1.6	39.1 ± 1.8
AL-G10	74.4 ± 7.9	73.4 ± 0.2	68.2 ± 0.1	29.7 ± 4.6	12.0 ± 2.4
AL-H10	80.1 ± 2.0	57.1 ± 2.5	50.5 ± 0.7	26.7 ± 0.9	11.4 ± 0.1
DOTAP:chol	78.4 ± 1.5	73.1 ± 1.1	65.3 ± 0.1	22.0 ± 0.2	17.4 ± 1.1

2.3 Structure-Activity-Relationship

The SAR was done for aminolipids AL-A10, AL-A12, AL-B10, AL-B12, and DOTAP:chol. A total of three independent measurements with three replicates each was done to obtain the data necessary for statistical evaluation. Statistical evaluation was carried out using Origin 2018 Pro (see Materials and Methods).

2.3.1 Results of the Structure-Activity-Relationship experiments

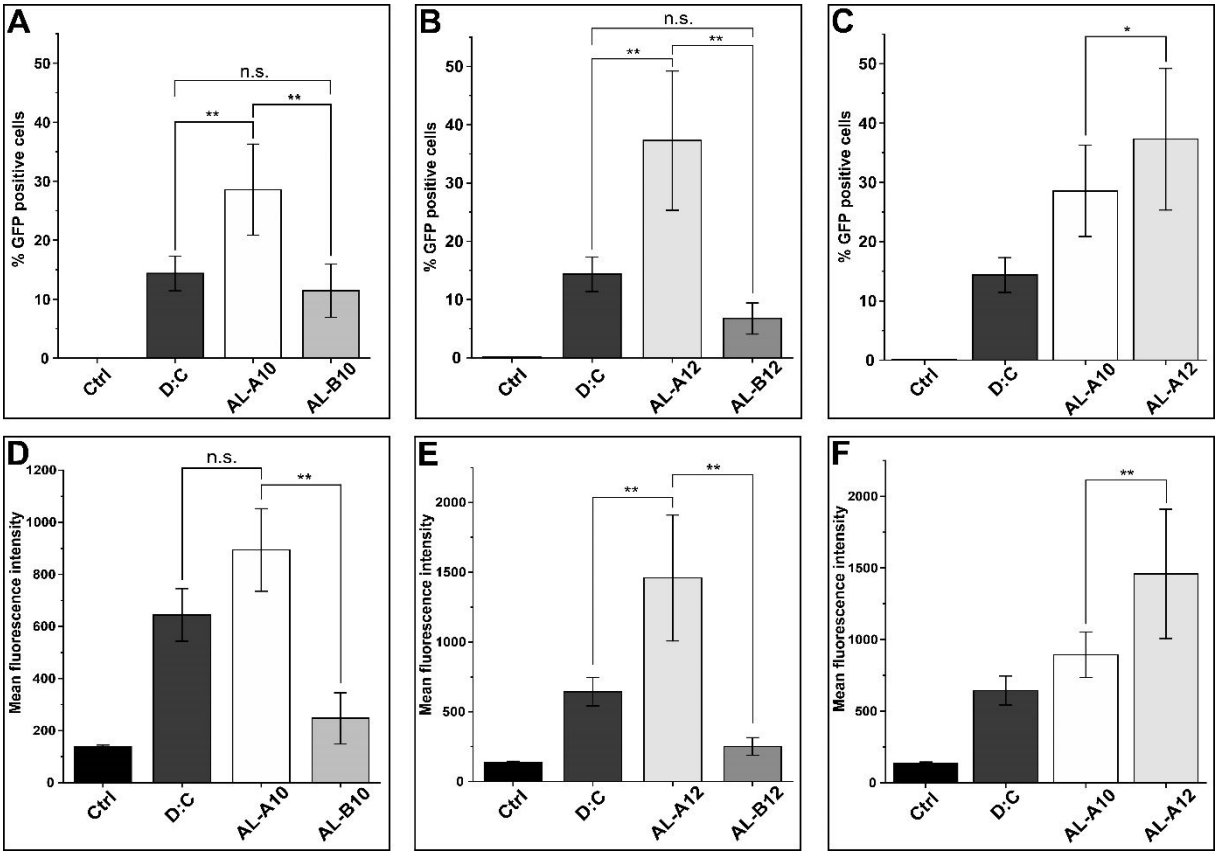

Figure S37: Structure-Activity-Relationship for the most promising aminolipids AL-A10, AL-A12, AL-B10, AL-B12, as well as DOTAP:chol. The graph shows the influence of the headgroup on transfection efficiency (A, B) and transgene expression (D, E) when the tail length stays constant and the influence of the tail length on transfection efficiency (C) and transgene expression (F) when the headgroup stays constant. Methoxy headgroups are superior to hydroxy headgroups ($p < 0.001$) and C_{12} tails are superior to C_{10} tails in both, transfection efficiency ($p < 0.1$) and transgene expression ($p < 0.001$). The data were obtained from three independent measurements with three replicates each. * $p < 0.1$, ** $p < 0.001$.

2.3.2 ANOVA Tables for GFP (p < 0.1)

Table S4: Descriptive statistics for the one way ANOVA for GFP (p < 0.1). AL 1 corresponds to AL-A10, AL 2 corresponds to AL-A12, AL 3 corresponds to AL-B10, and AL 4 corresponds to AL-B12.

	N Analysis	N Missing	Mean	Standard Deviation	SE of Mean
DOTAP/Chol 1 ug	8	1	6.0	0.7	0.3
AL 1 1 ug	9	0	7.9	1.9	0.6
AL 2 1 ug	9	0	11.2	4.2	1.4
AL 3 1 ug	9	0	4.7	2.0	0.7
AL 4 1 ug	9	0	7.6	3.1	1.0
DOTAP/Chol 2 ug	9	0	14.3	3.1	1.0
AL 1 2 ug	9	0	28.6	8.2	2.7
AL 2 2 ug	9	0	37.3	12.7	4.2
AL 3 2 ug	9	0	11.5	4.8	1.6
AL 4 2 ug	9	0	6.8	2.8	0.9

Table S5: Overall ANOVA table for the one way ANOVA for GFP (p < 0.1). Null Hypothesis: The means of all levels are equal. Alternative Hypothesis: The means of one or more levels are different. At the 0.1 level, the population means are significantly different.

	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	9	9374.4	1041.6	33.9	0
Error	79	2428.6	30.7		
Total	88	11803.0			

Table S6: Fit statistics for the one way ANOVA for GFP (p < 0.1).

	R-Square	Coeff Var	Root MSE	Data Mean
	0.794	0.406	5.545	13.661

Table S7: Means comparison Bonferroni test for one way ANOVA for GFP (p < 0.1). Sig equals 1 indicates that the difference of the means is significant at the 0.1 level. Sig equals 0 indicates that the difference of the means is not significant at the 0.1 level. AL 1 corresponds to AL-A10, AL 2 corresponds to AL-A12, AL 3 corresponds to AL-B10, and AL 4 corresponds to AL-B12.

	MeanDiff	SEM	t Value	Prob	Alpha	Sig	LCL	UCL
AL 1 1 ug DOTAP/Chol 1 ug	1.9	2.7	0.7	1	0.1	0	-6.6	10.4
AL 2 1 ug DOTAP/Chol 1 ug	5.2	2.7	1.9	1	0.1	0	-3.4	13.7
AL 2 1 ug AL 1 1 ug	3.2	2.6	1.2	1	0.1	0	-5.0	11.5
AL 3 1 ug DOTAP/Chol 1 ug	-1.3	2.7	-0.5	1	0.1	0	-9.8	7.2
AL 3 1 ug AL 1 1 ug	-3.2	2.6	-1.2	1	0.1	0	-11.5	5.0
AL 3 1 ug AL 2 1 ug	-6.5	2.6	-2.5	0.7	0.1	0	-14.7	1.8
AL 4 1 ug DOTAP/Chol 1 ug	1.6	2.7	0.6	1	0.1	0	-6.9	10.1
AL 4 1 ug AL 1 1 ug	-0.3	2.6	-0.1	1	0.1	0	-8.6	7.9
AL 4 1 ug AL 2 1 ug	-3.6	2.6	-1.4	1	0.1	0	-11.8	4.7
AL 4 1 ug AL 3 1 ug	2.9	2.6	1.1	1	0.1	0	-5.4	11.2
DOTAP/Chol 2 ug DOTAP/Chol 1ug	8.3	2.7	3.1	0.1	0.1	0	-0.2	16.9
DOTAP/Chol 2 ug AL 1 1 ug	6.4	2.6	2.5	0.7	0.1	0	-1.8	14.7
DOTAP/Chol 2 ug AL 2 1 ug	3.2	2.6	1.2	1	0.1	0	-5.1	11.5
DOTAP/Chol 2 ug AL 3 1 ug	9.7	2.6	3.7	0.0	0.1	1	1.4	17.9
DOTAP/Chol 2 ug AL 4 1 ug	6.8	2.6	2.6	0.5	0.1	0	-1.5	15.0
AL 1 2 ug DOTAP/Chol 1 ug	22.6	2.7	8.4	7.4E-11	0.1	1	14.0	31.1
AL 1 2 ug AL 1 1 ug	20.6	2.6	7.9	6.2E-10	0.1	1	12.4	28.9
AL 1 2 ug AL 2 1 ug	17.4	2.6	6.7	1.5E-7	0.1	1	9.1	25.7

AL 1 2 ug AL 3 1 ug	23.9	2.6	9.1	2.4E-12	0.1	1	15.6	32.2
AL 1 2 ug AL 4 1 ug	21.0	2.6	8.0	3.5E-10	0.1	1	12.7	29.2
AL 1 2 ug DOTAP/Chol 2 ug	14.2	2.6	5.4	2.6E-5	0.1	1	6.0	22.5
AL 2 2 ug DOTAP/Chol 1 ug	31.3	2.7	11.6	4.3E-17	0.1	1	22.8	39.8
AL 2 2 ug AL 1 1 ug	29.4	2.6	11.2	2.2-16	0.1	1	21.1	37.6
AL 2 2 ug AL 2 1 ug	26.1	2.6	10.0	5.1E-14	0.1	1	17.9	34.4
AL 2 2 ug AL 3 1 ug	32.6	2.6	12.5	1.1E-18	0.1	1	24.3	40.9
AL 2 2 ug AL 4 1 ug	29.7	2.6	11.4	1.3E-16	0.1	1	21.4	38.0
AL 2 2 ug DOTAP/Chol 2 ug	22.9	2.6	8.8	1.2E-11	0.1	1	14.7	31.2
AL 2 2 ug AL 1 2 ug	8.7	2.6	3.3	0.1	0.1	1	0.5	17.0
AL 3 2 ug DOTAP/Chol 1 ug	5.4	2.7	2.0	1	0.1	0	-3.1	14.0
AL 3 2 ug AL 1 1 ug	3.5	2.6	1.4	1	0.1	0	-4.7	11.8
AL 3 2 ug AL 2 1 ug	0.3	2.6	0.1	1	0.1	0	-8.0	8.6
AL 3 2 ug AL 3 1 ug	6.8	2.6	2.6	0.5	0.1	0	-1.5	15.0
AL 3 2 ug AL 4 1 ug	3.9	2.6	1.5	1	0.1	0	-4.4	12.1
AL 3 2 ug DOTAP/Chol 2 ug	-2.9	2.6	-1.1	1	0.1	0	-11.2	5.4
AL 3 2 ug AL 1 2 ug	-17.1	2.6	-6.6	2.4E-7	0.1	1	-25.4	-8.9
AL 3 2 ug AL 2 2 ug	-25.8	2.6	-9.9	8.4E-14	0.1	1	-34.1	-17.6
AL 4 2 ug DOTAP/Chol 1 ug	0.8	2.7	0.3	1	0.1	0	-7.8	9.3
AL 4 2 ug AL 1 1 ug	-1.2	2.6	-0.5	1	0.1	0	-9.4	7.1
AL 4 2 ug AL 2 1 ug	-4.4	2.6	-1.7	1	0.1	0	-12.7	3.9
AL 4 2 ug AL 3 1 ug	2.1	2.6	0.8	1	0.1	0	-6.2	10.3
AL 4 2 ug AL 4 1 ug	-0.8	2.6	-0.3	1	0.1	0	-9.1	7.4
AL 4 2 ug DOTAP/Chol 2 ug	-7.6	2.6	-2.9	0.2	0.1	0	-15.9	0.7
AL 4 2 ug AL 1 2 ug	-21.8	2.6	-8.3	8.4E-11	0.1	1	-30.1	-13.6
AL 4 2 ug AL 2 2 ug	-30.5	2.6	-11.7	3.1E-17	0.1	1	-38.8	-22.3
AL 4 2 ug AL 3 2 ug	-4.7	2.6	-1.8	1	0.1	0	-13.0	3.6

2.3.3 ANOVA Table for GFP (p < 0.001)

Table S8: Descriptive statistics for the one way ANOVA for GFP (p < 0.001). AL 1 corresponds to AL-A10, AL 2 corresponds to AL-A12, AL 3 corresponds to AL-B10, and AL 4 corresponds to AL-B12.

	N Analysis	N Missing	Mean	Standard Deviation	SE of Mean
DOTAP/Chol 1 ug	8	1	6.0	0.7	0.3
AL 1 1 ug	9	0	7.9	1.9	0.6
AL 2 1 ug	9	0	11.2	4.2	1.4
AL 3 1 ug	9	0	4.7	2.0	0.7
AL 4 1 ug	9	0	7.6	3.1	1.0
DOTAP/Chol 2 ug	9	0	14.3	3.1	1.0
AL 1 2 ug	9	0	28.6	8.2	2.7
AL 2 2 ug	9	0	37.3	12.7	4.2
AL 3 2 ug	9	0	11.5	4.8	1.6
AL 4 2 ug	9	0	6.8	2.8	1.0

Table S9: Overall ANOVA table for the one way ANOVA for GFP (p < 0.001). Null Hypothesis: The means of all levels are equal. Alternative Hypothesis: The means of one or more levels are different. At the 0.001 level, the population means are significantly different.

	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	9	9374.4	1041.6	33.9	0
Error	79	2428.6	30.7		
Total	88	11803.0			

Table S10: Fit statistics for the one way ANOVA for GFP (p < 0.001).

	R-Square	Coeff Var	Root MSE	Data Mean

	0.794	0.406	5.545	13.661

Table S11: Means comparison Bonferroni test for one way ANOVA for GFP (p < 0.001). Sig equals 1 indicates that the difference of the means is significant at the 0.001 level. Sig equals 0 indicates that the difference of the means is not significant at the 0.001 level. AL 1 corresponds to AL-A10, AL 2 corresponds to AL-A12, AL 3 corresponds to AL-B10, and AL 4 corresponds to AL-B12.

	MeanDiff	SEM	t Value	Prob	Alpha	Sig	LCL	UCL
AL 1 1 ug DOTAP/Chol 1 ug	1.9	2.7	0.7	1	0.001	0	-10.2	14.1
AL 2 1 ug DOTAP/Chol 1 ug	5.2	2.7	1.9	1	0.001	0	-7.0	17.3
AL 2 1 ug AL 1 1 ug	3.2	2.6	1.2	1	0.001	0	-8.6	15.0
AL 3 1 ug DOTAP/Chol 1 ug	-1.3	2.7	-0.5	1	0.001	0	-13.5	10.8
AL 3 1 ug AL 1 1 ug	-3.2	2.6	-1.2	1	0.001	0	-15.0	8.6
AL 3 1 ug AL 2 1 ug	-6.5	2.6	-2.5	0.7	0.001	0	-18.3	5.3
AL 4 1 ug DOTAP/Chol 1 ug	1.6	2.7	0.6	1	0.001	0	-10.6	13.7
AL 4 1 ug AL 1 1 ug	-0.3	2.6	-0.1	1	0.001	0	-12.1	11.5
AL 4 1 ug AL 2 1 ug	-3.6	2.6	-1.4	1	0.001	0	-15.4	8.2
AL 4 1 ug AL 3 1 ug	2.9	2.6	1.1	1	0.001	0	-8.9	14.7
DOTAP/Chol 2 ug DOTAP/Chol 1ug	8.3	2.7	3.1	0.1	0.001	0	-3.8	20.5
DOTAP/Chol 2 ug AL 1 1 ug	6.4	2.6	2.5	0.7	0.001	0	-5.4	18.2
DOTAP/Chol 2 ug AL 2 1 ug	3.2	2.6	1.2	1	0.001	0	-8.6	15.0
DOTAP/Chol 2 ug AL 3 1 ug	9.7	2.6	3.7	0.0	0.001	0	-2.1	21.5
DOTAP/Chol 2 ug AL 4 1 ug	6.8	2.6	2.6	0.5	0.001	0	-5.0	18.5
AL 1 2 ug DOTAP/Chol 1 ug	22.6	2.7	8.4	7.4E-11	0.001	1	10.4	34.7
AL 1 2 ug AL 1 1 ug	20.6	2.6	7.9	6.2E-10	0.001	1	8.9	32.4
AL 1 2 ug AL 2 1 ug	17.4	2.6	6.7	1.5E-7	0.001	1	5.6	29.2
AL 1 2 ug AL 3 1 ug	23.9	2.6	9.1	2.4E-12	0.001	1	12.1	35.7
AL 1 2 ug AL 4 1 ug	21.0	2.6	8.0	3.5E-10	0.001	1	9.2	32.8
AL 1 2 ug DOTAP/Chol 2 ug	14.2	2.6	5.4	2.6E-5	0.001	1	2.4	26.0
AL 2 2 ug DOTAP/Chol 1 ug	31.3	2.7	11.6	4.3E-17	0.001	1	19.1	43.4
AL 2 2 ug AL 1 1 ug	29.4	2.6	11.2	2.2E-16	0.001	1	17.6	41.1
AL 2 2 ug AL 2 1 ug	26.1	2.6	10.0	5.1E-14	0.001	1	14.3	37.9
AL 2 2 ug AL 3 1 ug	32.6	2.6	12.5	1.1E-18	0.001	1	20.8	44.4
AL 2 2 ug AL 4 1 ug	29.7	2.6	11.4	1.3E-16	0.001	1	17.9	41.5
AL 2 2 ug DOTAP/Chol 2 ug	22.9	2.6	8.8	1.2E-11	0.001	1	11.2	34.7
AL 2 2 ug AL 1 2 ug	8.7	2.6	3.3	0.1	0.001	0	-3.1	20.5
AL 3 2 ug DOTAP/Chol 1 ug	5.4	2.7	2.0	1	0.001	0	-6.7	17.6
AL 3 2 ug AL 1 1 ug	3.5	2.6	1.4	1	0.001	0	-8.3	15.3
AL 3 2 ug AL 2 1 ug	0.3	2.6	0.1	1	0.001	0	-11.5	12.1
AL 3 2 ug AL 3 1 ug	6.8	2.6	2.6	0.5	0.001	0	-5.0	18.6
AL 3 2 ug AL 4 1 ug	3.9	2.6	1.5	1	0.001	0	-7.9	15.7
AL 3 2 ug DOTAP/Chol 2 ug	-2.9	2.6	-1.1	1	0.001	0	-14.7	8.9
AL 3 2 ug AL 1 2 ug	-17.1	2.6	-6.6	2.4E-7	0.001	1	-28.9	-5.3
AL 3 2 ug AL 2 2 ug	-25.8	2.6	-9.9	8.4E-14	0.001	1	-37.6	-14.0
AL 4 2 ug DOTAP/Chol 1 ug	0.8	2.7	0.28	1	0.001	0	-11.4	12.9
AL 4 2 ug AL 1 1 ug	-1.2	2.6	-0.5	1	0.001	0	-13.0	10.6
AL 4 2 ug AL 2 1 ug	-4.4	2.6	-1.7	1	0.001	0	-16.2	7.4
AL 4 2 ug AL 3 1 ug	2.1	2.6	0.8	1	0.001	0	-9.7	13.9
AL 4 2 ug AL 4 1 ug	-0.8	2.6	-0.3	1	0.001	0	-12.6	11.0
AL 4 2 ug DOTAP/Chol 2 ug	-7.6	2.6	-2.9	0.2	0.001	0	-19.4	4.2
AL 4 2 ug AL 1 2 ug	-21.8	2.6	-8.4	8.3E-11	0.001	1	-33.6	-10.0
AL 4 2 ug AL 2 2 ug	-30.5	2.6	-11.7	3.1E-17	0.001	1	-42.3	-18.7
AL 4 2 ug AL 3 2 ug	-4.7	2.6	-1.8	1	0.001	0	-16.5	7.1

2.3.4 ANOVA Table for MFI (p < 0.1)

Table S12: Descriptive statistics for the one way ANOVA for MFI (p < 0.1). AL 1 corresponds to AL-A10, AL 2 corresponds to AL-A12, AL 3 corresponds to AL-B10, and AL 4 corresponds to AL-B12.

	N Analysis	N Missing	Mean	Standard Deviation	SE of Mean
DOTAP/Chol 1 ug	8	1	417.5	21.4	7.6
AL 1 1 ug	9	0	409.4	40.0	13.3
AL 2 1 ug	9	0	623.7	152.2	50.5
AL 3 1 ug	8	1	333.3	23.6	8.4
AL 4 1 ug	9	0	320.8	78.8	26.3
DOTAP/Chol 2 ug	9	0	644.4	107.2	35.7
AL 1 2 ug	9	0	893.9	167.7	55.9
AL 2 2 ug	9	0	1459.3	478.0	159.3
AL 3 2 ug	9	0	247.3	103.9	34.6
AL 4 2 ug	9	0	250.8	67.1	22.4

Table S13: Overall ANOVA table for the one way ANOVA for MFI (p < 0.1). Null Hypothesis: The means of all levels are equal. Alternative Hypothesis: The means of one or more levels are different. At the 0.1 level, the population means are significantly different.

	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	9	1.1E7	1.3E6	39.2	0
Error	78	2.5E6	32335.8		
Total	87	1.4E7			

Table S14: Fit statistics for the one way ANOVA for MFI (p < 0.1).

	R-Square	Coeff Var	Root MSE	Data Mean
	0.819	0.319	179.822	564.239

Table S15: Means comparison Bonferroni test for one way ANOVA for MFI (p < 0.1). Sig equals 1 indicates that the difference of the means is significant at the 0.1 level. Sig equals 0 indicates that the difference of the means is not significant at the 0.1 level. AL 1 corresponds to AL-A10, AL 2 corresponds to AL-A12, AL 3 corresponds to AL-B10, and AL 4 corresponds to AL-B12.

	MeanDiff	SEM	t Value	Prob	Alpha	Sig	LCL	UCL
AL 1 1 ug DOTAP/Chol 1 ug	-8.1	87.4	-0.1	1	0.1	0	-284.5	268.4
AL 2 1 ug DOTAP/Chol 1 ug	206.2	87.4	2.4	0.9	0.1	0	-70.3	482.6
AL 2 1 ug AL 1 1 ug	214.2	84.8	2.5	0.6	0.1	0	-53.9	482.4
AL 3 1 ug DOTAP/Chol 1 ug	-84.3	89.9	-0.9	1	0.1	0	-368.7	200.2
AL 3 1 ug AL 1 1 ug	-76.2	87.4	-0.9	1	0.1	0	-352.6	200.2
AL 3 1 ug AL 2 1 ug	-290.4	87.4	-3.3	0.1	0.1	1	-566.8	-14.0
AL 4 1 ug DOTAP/Chol 1 ug	-96.7	87.4	-1.1	1	0.1	0	-373.1	179.7
AL 4 1 ug AL 1 1 ug	-88.7	84.8	-1.0	1	0.1	0	-356.8	179.5
AL 4 1 ug AL 2 1 ug	-302.9	84.8	-3.6	0.0	0.1	1	-571.1	-34.7
AL 4 1 ug AL 3 1 ug	-12.5	87.4	-0.1	1	0.1	0	-288.9	263.9
DOTAP/Chol 2 ug DOTAP/Chol 1 ug	226.9	87.4	2.6	0.5	0.1	0	-49.5	503.4
DOTAP/Chol 2 ug AL 1 1 ug	235.0	84.8	2.8	0.3	0.1	0	-33.2	503.2
DOTAP/Chol 2 ug AL 2 1 ug	20.8	84.8	0.2	1	0.1	0	-247.4	288.9
DOTAP/Chol 2 ug AL 3 1 ug	311.2	87.4	3.6	0.0	0.1	1	34.8	587.6
DOTAP/Chol 2 ug AL 4 1 ug	323.7	84.8	3.8	0.0	0.1	1	55.5	591.8
AL 1 2 ug DOTAP/Chol 1 ug	476.4	87.4	5.5	2.5E-5	0.1	1	200.0	752.8
AL 1 2 ug AL 1 1 ug	484.4	84.8	5.7	8.6E-6	0.1	1	216.3	752.6

AL 1 2 ug AL 2 1 ug	270.2	84.8	3.2	0.1	0.1	1	2.1	538.4
AL 1 2 ug AL 3 1 ug	560.6	87.4	6.4	4.5E-7	0.1	1	284.2	837.1
AL 1 2 ug AL 4 1 ug	573.1	84.8	6.8	1.0E-7	0.1	1	304.9	841.3
AL 1 2 ug DOTAP/Chol 2 ug	249.4	84.8	2.9	0.2	0.1	0	-18.7	517.6
AL 2 2 ug DOTAP/Chol 1 ug	1041.8	87.4	11.9	1.3E-17	0.1	1	765.4	1318.3
AL 2 2 ug AL 1 1 ug	1049.9	84.8	12.4	1.9E-18	0.1	1	781.7	1318.1
AL 2 2 ug AL 2 1 ug	835.7	84.8	9.9	1.1E-13	0.1	1	567.5	1103.8
AL 2 2 ug AL 3 1 ug	1126.1	87.4	12.9	2.3E-19	0.1	1	849.7	1402.5
AL 2 2 ug AL 4 1 ug	1138.6	84.8	13.4	2.5E-20	0.1	1	870.4	1406.7
AL 2 2 ug DOTAP/Chol 2 ug	814.9	84.8	9.6	3.2E-13	0.1	1	546.7	1083.1
AL 2 2 ug AL 1 2 ug	565.4	84.8	6.7	1.5E-7	0.1	1	297.3	833.6
AL 3 2 ug DOTAP/Chol 1 ug	-170.2	87.4	-1.9	1	0.1	0	-446.6	106.3
AL 3 2 ug AL 1 1 ug	-162.1	84.8	-1.9	1	0.1	0	-430.3	106.1
AL 3 2 ug AL 2 1 ug	-376.3	84.8	-4.4	0.0	0.1	1	-644.5	-108.2
AL 3 2 ug AL 3 1 ug	-85.9	87.4	-1.0	1	0.1	0	-362.3	190.5
AL 3 2 ug AL 4 1 ug	-73.4	84.8	-0.9	1	0.1	0	-341.6	194.7
AL 3 2 ug DOTAP/Chol 2 ug	-397.1	84.8	-4.7	5.3E-4	0.1	1	-665.3	-128.9
AL 3 2 ug AL 1 2 ug	-646.6	84.8	-7.6	2.2E-9	0.1	1	-914.7	-378.4
AL 3 2 ug AL 2 2 ug	-1212.0	84.8	-14.3	7.6E-22	0.1	1	-1480.2	-943.8
AL 4 2 ug DOTAP/Chol 1 ug	-166.7	87.4	-1.9	1	0.1	0	-443.1	109.7
AL 4 2 ug AL 1 1 ug	-158.7	84.8	-1.9	1	0.1	0	-426.8	109.5
AL 4 2 ug AL 2 1 ug	-372.9	84.8	-4.4	0.0	0.1	1	-641.1	-104.7
AL 4 2 ug AL 3 1 ug	-82.5	87.4	-0.9	1	0.1	0	-358.9	193.9
AL 4 2 ug AL 4 1 ug	-70.0	84.8	-0.8	1	0.1	0	-338.2	198.2
AL 4 2 ug DOTAP/Chol 2 ug	-393.7	84.8	-4.6	6.1E-4	0.1	1	-661.8	-125.5
AL 4 2 ug AL 1 2 ug	-643.1	84.8	-7.6	2.7E-9	0.1	1	-911.3	-374.9
AL 4 2 ug AL 2 2 ug	-1208.6	84.8	-14.3	9.0E-22	0.1	1	-1476.7	-940.4
AL 4 2 ug AL 3 2 ug	3.4	84.8	0.0	1	0.1	0	-264.7	271.6

2.3.5 ANOVA Table for MFI ($p < 0.001$)

Table S16: Descriptive statistics for the one way ANOVA for MFI ($p < 0.001$). AL 1 corresponds to AL-A10, AL 2 corresponds to AL-A12, AL 3 corresponds to AL-B10, and AL 4 corresponds to AL-B12.

	N Analysis	N Missing	Mean	Standard Deviation	SE of Mean
DOTAP/Chol 1 ug	8	1	417.5	21.4	7.6
AL 1 1 ug	9	0	409.4	40.0	13.3
AL 2 1 ug	9	0	623.6	152.2	50.7
AL 3 1 ug	8	1	333.3	23.6	8.3
AL 4 1 ug	9	0	320.8	78.8	26.3
DOTAP/Chol 2 ug	9	0	644.4	107.2	35.7
AL 1 2 ug	9	0	893.9	167.7	55.9
AL 2 2 ug	9	0	1459.3	478.0	159.3
AL 3 2 ug	9	0	247.3	103.9	34.6
AL 4 2 ug	9	0	250.8	67.1	22.4

Table S17: Overall ANOVA table for the one way ANOVA for MFI ($p < 0.001$). Null Hypothesis: The means of all levels are equal. Alternative Hypothesis: The means of one or more levels are different. At the 0.001 level, the population means are significantly different.

	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	9	1.1E7	1.3E6	39.2	0
Error	78	2.5E6	32335.8		
Total	87	1.4E7			

Table S18: Fit statistics for the one way ANOVA for MFI (p < 0.001).

	R-Square	Coeff Var	Root MSE	Data Mean
	0.819	0.319	179.822	564.239

Table S19: Means comparison Bonferroni test for one way ANOVA for MFI (p < 0.001). Sig equals 1 indicates that the difference of the means is significant at the 0.001 level. Sig equals 0 indicates that the difference of the means is not significant at the 0.001 level. AL 1 corresponds to AL-A10, AL 2 corresponds to AL-A12, AL 3 corresponds to AL-B10, and AL 4 corresponds to AL-B12.

	MeanDiff	SEM	t Value	Prob	Alpha	Sig	LCL	UCL
AL 1 1 ug DOTAP/Chol 1 ug	-8.1	87.4	-0.1	1	0.001	0	-402.5	386.4
AL 2 1 ug DOTAP/Chol 1 ug	206.2	87.4	2.4	0.9	0.001	0	-188.2	600.6
AL 2 1 ug AL 1 1 ug	214.2	84.8	2.5	0.6	0.001	0	-168.4	596.9
AL 3 1 ug DOTAP/Chol 1 ug	-84.3	89.9	-0.9	1	0.001	0	-490.1	321.6
AL 3 1 ug AL 1 1 ug	-76.2	87.4	-0.9	1	0.001	0	-470.6	318.2
AL 3 1 ug AL 2 1 ug	-290.4	87.4	-3.3	0.1	0.001	0	-684.8	104.0
AL 4 1 ug DOTAP/Chol 1 ug	-96.7	87.4	-1.1	1	0.001	0	-491.1	297.7
AL 4 1 ug AL 1 1 ug	-88.7	84.8	-1.0	1	0.001	0	-471.3	294.0
AL 4 1 ug AL 2 1 ug	-302.9	84.8	-3.6	0.0	0.001	0	-685.5	79.7
AL 4 1 ug AL 3 1 ug	-12.5	87.4	-0.1	1	0.001	0	-406.9	381.9
DOTAP/Chol 2 ug DOTAP/Chol 1 ug	226.9	87.4	2.6	0.5	0.001	0	-167.5	621.4
DOTAP/Chol 2 ug AL 1 1 ug	235.0	84.8	2.8	0.3	0.001	0	-147.6	617.6
DOTAP/Chol 2 ug AL 2 1 ug	20.8	84.8	0.2	1	0.001	0	-361.9	403.4
DOTAP/Chol 2 ug AL 3 1 ug	311.2	87.4	3.6	0.0	0.001	0	-83.2	705.6
DOTAP/Chol 2 ug AL 4 1 ug	323.7	84.8	3.8	0.0	0.001	0	-59.0	706.3
AL 1 2 ug DOTAP/Chol 1 ug	476.4	87.4	5.5	2.5E-5	0.001	1	82.0	870.8
AL 1 2 ug AL 1 1 ug	484.4	84.8	5.7	8.6E-6	0.001	1	101.8	867.1
AL 1 2 ug AL 2 1 ug	270.2	84.8	3.2	0.1	0.001	0	-112.4	652.9
AL 1 2 ug AL 3 1 ug	560.6	87.4	6.4	4.5E-7	0.001	1	166.2	955.1
AL 1 2 ug AL 4 1 ug	573.1	84.8	6.8	1.0E-7	0.001	1	190.5	955.7
AL 1 2 ug DOTAP/Chol 2 ug	249.4	84.8	2.9	0.2	0.001	0	-133.2	632.1
AL 2 2 ug DOTAP/Chol 1 ug	1041.8	87.4	11.9	1.3E-17	0.001	1	647.4	1436.2
AL 2 2 ug AL 1 1 ug	1049.9	84.8	12.4	1.9E-18	0.001	1	667.3	1432.5
AL 2 2 ug AL 2 1 ug	835.7	84.8	9.9	1.1E-13	0.001	1	453.0	1218.3
AL 2 2 ug AL 3 1 ug	1126.1	87.4	12.9	2.3E-19	0.001	1	731.7	1520.5
AL 2 2 ug AL 4 1 ug	1138.6	84.8	13.4	2.5E-20	0.001	1	755.9	1521.2
AL 2 2 ug DOTAP/Chol 2 ug	814.9	84.8	9.6	3.2E-13	0.001	1	432.3	1197.5
AL 2 2 ug AL 1 2 ug	565.4	84.8	6.7	1.5E-7	0.001	1	182.8	948.1
AL 3 2 ug DOTAP/Chol 1 ug	-170.2	87.4	-1.9	1	0.001	0	-564.6	224.2
AL 3 2 ug AL 1 1 ug	-162.1	84.8	-1.9	1	0.001	0	-544.7	220.5
AL 3 2 ug AL 2 1 ug	-376.3	84.8	-4.4	0.0	0.001	0	-759.0	6.3
AL 3 2 ug AL 3 1 ug	-85.9	87.4	-1.0	1	0.001	0	-480.3	308.5
AL 3 2 ug AL 4 1 ug	-73.4	84.8	-0.9	1	0.001	0	-456.1	309.2
AL 3 2 ug DOTAP/Chol 2 ug	-397.1	84.8	-4.7	5.3E-4	0.001	1	-779.7	-14.5
AL 3 2 ug AL 1 2 ug	-646.6	84.8	-7.6	2.2E-9	0.001	1	-1029.2	-263.9
AL 3 2 ug AL 2 2 ug	-1212.0	84.8	-14.3	7.6E-22	0.001	1	-1594.6	-829.4
AL 4 2 ug DOTAP/Chol 1 ug	-166.7	87.4	-1.9	1	0.001	0	-561.1	227.7
AL 4 2 ug AL 1 1 ug	-158.7	84.8	-1.9	1	0.001	0	-541.3	224.0
AL 4 2 ug AL 2 1 ug	-372.9	84.8	-4.4	0.0	0.001	0	-755.5	9.7
AL 4 2 ug AL 3 1 ug	-82.5	87.4	-0.9	1	0.001	0	-476.9	311.9
AL 4 2 ug AL 4 1 ug	-70.0	84.8	-0.8	1	0.001	0	-452.6	312.6
AL 4 2 ug DOTAP/Chol 2 ug	-393.7	84.8	-4.6	6.1E-4	0.001	1	-776.3	-11.0
AL 4 2 ug AL 1 2 ug	-643.1	84.8	-7.6	2.7E-9	0.001	1	-1025.7	-260.5
AL 4 2 ug AL 2 2 ug	-1208.6	84.8	-14.3	9.0E-22	0.001	1	-1591.2	-825.9
AL 4 2 ug AL 3 2 ug	3.4	84.8	0.0	1	0.001	0	-379.2	386.1

2.3.6 Outlier Detection

Table S 20: Outlier detection for GFP at 1µg/mL DNA and a lipid-to-DNA ratio of 1. Outliers (red) were defined as values larger than the upper fence or lower than the lower fence and were excluded from analysis.Upper fence is defined by Q3 + 1.5x interquartile range (IQR) and lower fence is defined by Q1 – 1.5x IQR. Only one oulier could be identified.

Control		DOTAP:chol		AL-A10		AL-B10		AL-A12		AL-B12	
0.12	0.12	6.52	6.52	8.28	8.28	4.90	4.90	5.25	5.25	4.05	4.05
0.06	0.06	4.74	4.74	6.14	6.14	4.05	4.05	7.18	7.18	8.42	8.42
0.12	0.12	6.75	6.75	8.17	8.17	9.01	9.01	15.20	15.20	9.65	9.65
		5.98	5.98	11.50	11.50	4.28	4.28	6.34	6.34	6.34	6.34
		5.26	5.26	6.72	6.72	2.27	2.27	12.50	12.50	4.83	4.83
		6.52	6.52	7.95	7.95	5.76	5.76	15.60	15.60	9.67	9.67
		10.5		8.32	8.32	3.07	3.07	9.67	9.67	4.88	4.88
		5.78	5.78	5.00	5.00	3.16	3.16	12.60	12.60	6.66	6.66
		6.54	6.54	9.23	9.23	5.66	5.66	16.10	16.10	13.8	13.8
Q1	0.06	**Q1**	5.52	**Q1**	6.43	**Q1**	3.12	**Q1**	6.76	**Q1**	4.86
Q3	0.12	**Q3**	6.65	**Q3**	8.78	**Q3**	5.71	**Q3**	15.4	**Q3**	9.66
IQR	0.06	**IQR**	1.13	**IQR**	2.35	**IQR**	2.60	**IQR**	8.64	**IQR**	4.81
Upper Fence	0.21	**Upper Fence**	8.33	**Upper Fence**	12.29	**Upper Fence**	9.60	**Upper Fence**	28.36	**Upper Fence**	16.87
Lower Fence	-0.03	**Lower Fence**	3.83	**Lower Fence**	2.91	**Lower Fence**	-0.78	**Lower Fence**	-6.20	**Lower Fence**	-2.35
Median	0.12	**Median**	6.25	**Median**	8.17	**Median**	4.28	**Median**	12.5	**Median**	6.66
Average	0.10	**Average**	6.01	**Average**	7.92	**Average**	4.68	**Average**	11.16	**Average**	7.59
S.D.	0.03	**S.D.**	0.67	**S.D.**	1.76	**S.D.**	1.89	**S.D.**	3.95	**S.D.**	2.94
RSD	28.85	**RSD**	11.13	**RSD**	22.27	**RSD**	40.37	**RSD**	35.41	**RSD**	38.73

Table S21: Outlier detection for GFP at 2µg/mL DNA and a lipid-to-DNA ratio of 1. Outliers (red) were defined as values larger than the upper fence or lower than the lower fence and were excluded from analysis.Upper fence is defined by Q3 + 1.5x interquartile range (IQR) and lower fence is defined by Q1 – 1.5x IQR. No outlier could be identified.

Control		DOTAP:chol		AL-A10		AL-B10		AL-A12		AL-B12	
0.10	0.10	10.60	10.60	19.50	19.50	11.20	11.20	17.30	17.30	9.78	9.78
0.11	0.11	16.20	16.20	33.50	33.50	7.91	7.91	45.20	45.20	7.43	7.43
0.09	0.09	12.60	12.60	33.40	33.40	8.00	8.00	44.50	44.50	12.70	12.70
		10.60	10.60	16.70	16.70	7.92	7.92	22.00	22.00	3.99	3.99
		17.00	17.00	32.70	32.70	9.37	9.37	43.00	43.00	6.41	6.41
		16.30	16.30	37.10	37.10	8.74	8.74	53.00	53.00	4.93	4.93
		11.30	11.30	17.40	17.40	11.70	11.70	23.80	23.80	5.23	5.23
		19.10	19.10	32.80	32.80	22.20	22.20	44.60	44.60	5.91	5.91
		15.40	15.40	34.00	34.00	16.00	16.00	42.10	42.10	4.43	4.43
Q1	0.09	**Q1**	10.95	**Q1**	18.45	**Q1**	7.96	**Q1**	22.90	**Q1**	4.68
Q3	0.11	**Q3**	16.65	**Q3**	33.75	**Q3**	13.85	**Q3**	44.90	**Q3**	8.61
IQR	0.02	**IQR**	5.70	**IQR**	15.30	**IQR**	5.89	**IQR**	22.00	**IQR**	3.93
Upper Fence	0.14	**Upper Fence**	25.20	**Upper Fence**	56.70	**Upper Fence**	22.69	**Upper Fence**	77.90	**Upper Fence**	14.49
Lower Fence	0.06	**Lower Fence**	2.40	**Lower Fence**	-4.50	**Lower Fence**	-0.87	**Lower Fence**	-10.10	**Lower Fence**	-1.21
Median	0.10	**Median**	15.40	**Median**	32.80	**Median**	9.37	**Median**	43.00	**Median**	5.91
Average	0.10	**Average**	14.34	**Average**	28.57	**Average**	11.45	**Average**	37.28	**Average**	6.76
S.D.	0.01	**S.D.**	2.95	**S.D.**	7.69	**S.D.**	4.53	**S.D.**	11.95	**S.D.**	2.68
RSD	9.12	**RSD**	20.58	**RSD**	26.93	**RSD**	39.60	**RSD**	32.07	**RSD**	39.62

Table S22: Outlier detection for MFI at 1µg/mL DNA and a lipid-to-DNA ratio of 1. Outliers (red) were defined as values larger than the upper fence or lower than the lower fence and were excluded from analysis.Upper fence is defined by Q3 + 1.5x interquartile range (IQR) and lower fence is defined by Q1 – 1.5x IQR. Only two ouliers could be identified.

Control		DOTAP:chol		AL-A10		AL-B10		AL-A12		AL-B12	
182	182	409	409	379	379	333	333	424	424	294	294
127	127	398	398	395	395	354	354	451	451	290	290
147	147	461	461	426	426	420		819	819	328	328
		425	425	430	430	344	344	451	451	277	277
		401	401	477	477	313	313	582	582	291	291
		407	407	402	402	329	329	752	752	438	438
		596		392	392	291	291	714	714	224	224
		405	405	340	340	335	335	653	653	281	281
		434	434	444	444	367	367	767	767	464	464
Q1	127	**Q1**	403	**Q1**	385.5	**Q1**	321	**Q1**	451	**Q1**	279
Q3	182	**Q3**	447.5	**Q3**	437	**Q3**	360.5	**Q3**	759.5	**Q3**	383
IQR	55	**IQR**	44.5	**IQR**	51.5	**IQR**	39.5	**IQR**	308.5	**IQR**	104
Upper Fence	264.50	**Upper Fence**	514.25	**Upper Fence**	514.25	**Upper Fence**	419.75	**Upper Fence**	1222.25	**Upper Fence**	539.00
Lower Fence	44.50	**Lower Fence**	336.25	**Lower Fence**	308.25	**Lower Fence**	261.75	**Lower Fence**	-11.75	**Lower Fence**	123.00
Median	147	**Median**	408	**Median**	402	**Median**	334	**Median**	653	**Median**	291
Average	152.00	**Average**	417.50	**Average**	409.44	**Average**	333.25	**Average**	623.67	**Average**	320.78
S.D.	22.73	**S.D.**	20.04	**S.D.**	37.67	**S.D.**	22.08	**S.D.**	143.53	**S.D.**	74.33
RSD	14.95	**RSD**	4.80	**RSD**	9.20	**RSD**	6.63	**RSD**	23.01	**RSD**	23.17

Table S 23: Outlier detection for MFI at 2µg/mL DNA and a lipid-to-DNA ratio of 1. Outliers (red) were defined as values larger than the upper fence or lower than the lower fence and were excluded from analysis.Upper fence is defined by Q3 + 1.5x interquartile range (IQR) and lower fence is defined by Q1 – 1.5x IQR. No outlier could be identified.

Control		DOTAP:chol		AL-A10		AL-B10		AL-A12		AL-B12	
132	132	489	489	816	816	212	212	745	745	303	303
133	133	663	663	966	966	151	151	1972	1972	153	153
148	148	570	570	937	937	151	151	1658	1658	164	164
		531	531	694	694	308	308	968	968	238	238
		733	733	1063	1063	151	151	1492	1492	209	209
		753	753	956	956	164	164	2283	2283	253	253
		564	564	577	577	317	317	1155	1155	304	304
		774	774	1082	1082	427	427	1437	1437	354	354
		723	723	954	954	345	345	1424	1424	279	279
Q1	132	**Q1**	547.5	**Q1**	755	**Q1**	151	**Q1**	1061.5	**Q1**	186.5
Q3	148	**Q3**	743	**Q3**	1014.5	**Q3**	331	**Q3**	1815	**Q3**	303.5
IQR	16	**IQR**	195.5	**IQR**	259.5	**IQR**	180	**IQR**	753.5	**IQR**	117
Upper Fence	172.00	**Upper Fence**	1036.25	**Upper Fence**	1403.75	**Upper Fence**	601.00	**Upper Fence**	2945.25	**Upper Fence**	479.00
Lower Fence	108.00	**Lower Fence**	254.25	**Lower Fence**	365.75	**Lower Fence**	-119.00	**Lower Fence**	-68.75	**Lower Fence**	11.00
Median	133	**Median**	663	**Median**	954	**Median**	212	**Median**	1437	**Median**	253
Average	137.67	**Average**	644.44	**Average**	893.89	**Average**	247.33	**Average**	1459.33	**Average**	250.78
S.D.	7.32	**S.D.**	101.09	**S.D.**	158.10	**S.D.**	97.97	**S.D.**	450.66	**S.D.**	63.26
RSD	5.32	**RSD**	15.69	**RSD**	17.69	**RSD**	39.61	**RSD**	30.88	**RSD**	25.23